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SOME UNSOLVED PROBLEMS IN THE PARASITOLOGY OF AMEBIASIS*

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Since the discovery of *Endamoeba histolytica* by Lösch, in 1875, and the etiological relationship of this parasite to the infection known as amebiasis and the symptom-complex of that infection, amebic dysentery, by Kartulis (1886), Councilman and Lafleur (1891), Walker and Sellards (1913) and others, this parasite has received intensive study by many investigators and a large amount of most valuable data has been accumulated regarding the biology of the parasite and the epidemiology, pathology, symptomatology, diagnosis, prophylaxis and treatment of the infections caused by it. Although this is true, there still remain unsolved problems in the parasitology of amebiasis, three of which will be considered at this time.

I. *The Possible Existence of Avirulent Strains of Endamoeba histolytica.* The absence of definite symptomatology in many individuals harboring *E. histolytica* has led to the belief in certain quarters that there must exist strains of this parasite that are non-pathogenic and that such strains may live in the lumen of the host's intestine for indefinite periods. This belief was greatly stimulated and supported by the description by Brumpt (1928) of an ameba indistinguishable from *E. histolytica* in its morphology and life cycle which was little, if at all, pathogenic for kittens and non-pathogenic for man. Brumpt named this ameba *Entamoeba dispar* and stated that it had a wide geographic distribution in man and that it did not produce symptoms or lesions in the human host. In his opinion it is constantly confused with *E. histolytica* and is usually the species found in the so-called healthy carriers of the latter parasite.

Brumpt's observations regarding *E. dispar* have been confirmed by a few investigators although the vast majority of observers regard this species as identical with *E. histolytica* and it is not accepted by any of the English or American workers of large experience. It is not believed by the best authorities that mere differences in virulence between organ-

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isms otherwise identical constitute sufficient evidence upon which to base species, so that it follows that Brumpt's *E. dispar* can be regarded as no more than an avirulent strain of *E. histolytica* and the question is, whether such avirulent strains exist in nature or may be produced by cultivation or through passage in animals. The latter phase of the subject will be considered later in discussing the existence of strains of this parasite differing in virulence.

Perhaps the most convincing observations regarding the non-pathogenicity of an ameba which the observer regarded as identical with *E. dispar* are those of Simic. In 1931, this investigator published a paper describing his experiments with an ameba morphologically identical with *E. histolytica,* which he had obtained in cultures from 12 different individuals in Jugo-Slavia who had no history of dysentery and were apparently in good health. Seven of these strains were inoculated in cats, 6 cats being used for each strain, or 42 in all. Only 2 of these animals became infected and the amebae from these did not infect 3 other cats. The 2 strains that had infected the 2 cats were injected rectally into 4 human volunteers with negative results. In a later contribution (1931a) Simic records the successful infection of 6 human volunteers with this ameba and found that no illness resulted, although they were subjected to fatigue, changes in diet, and other conditions tending to decrease resistance. In a still later paper (1931b) he states that he was able to infect 7 young cats with the ameba with which the human volunteers were infected but that no symptoms occurred in these animals nor were macroscopic lesions present in the intestine even though the rectum contained numerous amebae. In the latest contribution of Simic that I have seen (1933) he states that he has infected puppies with the same ameba, that they presented no symptoms of the infection, and that autopsy revealed no macroscopic lesions in the large intestine. He states that the organism with which he has worked never ingests red blood corpuscles either in man or animals, one of the distinguishing features of *E. dispar,* according to Brumpt.

These observations of Simic are certainly very suggestive of the possible existence of an ameba identical in morphology and life history with *E. histolytica,* and parasitic in man, and apparently confirms Brumpt's observations regarding his *E. dispar.* Similar data have been reported by a few other observers but it is significant that this species has not been observed by English or American authorities of wide experience and that invariably the organisms observed by them having the morphology and characteristics of *E. dispar,* when inoculated into experimental animals, produced symptoms and lesions in those so inoculated. Thus Kessel (1928), working in China, and using amebae from symptomless carriers identical in morphology with Brumpt's *E. dispar,* found that inoculations

with them in kittens resulted in similar dysenteric symptoms and presented similar pathology to those infected with typical *E. histolytica* and he states "No evidence is produced for the conclusion of Brumpt that two species of amebae morphologically identical exist, one being pathogenic and the other non-pathogenic." Similar experiments to those of Kessel have been reported by numerous investigators with similar results.

Recently Hegner (1935) has reported results of experiments that apparently demonstrated that *E. histolytica* can live in the lumen of the intestine in monkeys without producing mascroscopic or microscopic lesions. This observer examined four *rhesus* monkeys that had passed cysts of *E. histolytica* for several weeks but in which no symptoms of the infection could be noted. At autopsy a most careful examination failed to reveal any macroscopic lesions of the infection and sections made at different levels of the large intestine were negative for the parasite. It is interesting to note that the strain used in these experiments was obtained from a fatal human case of amebic dysentery. Conceding that no microscopic lesions containing amebae existed in portions of the intestine not sectioned, these observations apparently prove that *E. histolytica* may exist as a commensal in the large intestine of certain monkeys for several weeks, but it far from demonstrates that there exist avirulent strains of this species which can live in the intestine of man without invading the tissues.

There are numerous records of the production of amebic dysentery in animals with strains of *E. histolytica* obtained from symptomless carriers and in Walker and Sellards' (1913) experiments upon human volunteers the four cases of amebic dysentery which occurred in their volunteers were produced by the ingestion of cysts obtained from carriers, two of whom had presented no symptoms of the infection, although under observation for months, and two of whom were convalescent carriers of the parasite. As showing the variation in pathology which may occur in experimental animals when a carrier strain of *E. histolytica* is employed, the observation by Faust (1932) is of interest, as he records a case in which material from a symptomless human carrier resulted in death from acute amebic dysentery in one dog, a mild enteritis in another and apparently failed to parasitise in the third animal. Such an observation indicates that one should be very cautions in drawing conclusions regarding the pathogenicity of any strain of this organism when passed through a few animals.

I have repeatedly produced dysenteric symptoms and ulcerative lesions in kittens with strains of *E. histolytica* from symptomless carriers in whom the infection had existed for weeks and months, and in one instance a cultural strain obtained from a presumably healthy carrier and cultivated for over four years was pathogenic to kittens at the end of that

period. Numerous other observations could be cited demonstrating that strains identical with *E. histolytica* in morphology, and obtained from symptomless carriers, have been proven to be pathogenic to animals when introduced into the rectum or the cysts fed by mouth.

In considering this problem it should be remembered that the absence of symptoms in amebic infection does not mean that the strain that is present is not virulent so far as the production of lesions is concerned. The observations of Kessel (1928), Hegner, Johnson and Stabler (1932), Craig and Kagy (1933) and others upon the lesions produced in experimental animals and of Councilman and Lafleur (1891), Dock (1891), Musgrave (1910), Bartlett (1917), Acton and Knowles (1924), Hiyeda and Suzuki (1932), Craig (1932–1934) and others upon the lesions found at autopsy in individuals dying from other causes and who had never presented any symptoms of diarrhea or dysentery sufficiently demonstrate the fact that a strain may be virulent enough to cause definite macroscopic lesions without producing clinical symptoms.

The recent observations of Meleney (1934) and Meleney and Frye (1934, 1935) are illuminating as regards the possible presence of *E. histolytica* in the tissues even though no macroscopic lesions are present. Meleney (1934) records the absence of necrosis of the tissues in such cases, although the glandular epithelium was missing in small areas, but *E. histolytica* were present in small areas throughout the tissues, while Meleney and Frye (1934) describe a case of a woman dying of chronic leukemia in whom no macroscopic or microscopic ulceration of the mucosa was detected but routine microscopical sections showed the parasites "beneath the epithelium at the bases of the mucosal glands, in the stroma of the mucosa, and in the tissue spaces, lymph follicles, lymph and blood follicles of the superficial portion of the sub-mucosa. The only tissue changes seen were loss of glandular epithelium and slight fraying of the superficial part of the stroma and the mucosa without definite necrosis." As suggested by Meleney (1934) these findings "may be the conditions that exist in some of the cases of amebiasis that present no clinical symptoms."

Despite the observations of Brumpt, Simic and others, the problem of the existence of avirulent strains of *E. histolytica* or of the existence of harmless species identical in life history must be regarded as still unsolved and it is my belief that the important question as to whether this parasite can live in the lumen of the bowel of man, under certain conditions, without producing lesions, or whether non-virulent strains exist can only be finally solved by observations upon man himself and not by the results of experiments on animals. It is unnecessary to dwell upon the importance of this problem in the prophylaxis and treatment of amebic infection and it deserves the most careful consideration and study.

II. *The Occurrence of Variations in Virulence in Strains of E. histolytica.* This problem embodies the possible existence of strains of *E. histolytica* of low or high virulence; whether such differences in virulence are fixed characteristics of such strains; or whether the virulence of a strain may be increased or decreased by cultivation and passage through experimental animals or man. This problem has received a considerable amount of attention from investigators and the literature concerning it is extensive. At this time it is only possible to consider a few of the most important contributions to its solution.

As long ago as 1914, Baetjer and Sellards recorded an increase in virulence in a strain of *E. histolytica* from a relapsing case of amebic dysentery after passage through kittens. Although no change occurred in the morphology of the parasite there was a decided increase in its virulence, the incubation period (the period elapsing between inoculation by direct injection into the cecum and the appearance of a bloody diarrhea) was shortened after the sixth passage, and the death of the animals occurred within a shorter period of time. These authors regarded the increase in virulence to be probably due to the bacteria present in the cecum rather than to an increase in virulence in the ameba. Cleveland and Sanders (1930) noted an increase in the virulence of experimental infections in kittens after the first and up to the fourth passage of strains of *E. histolytica* maintained in cultures for periods of between 460 and 540 days, although they admitted that the increase might have been due to the bacteria in the cultures rather than to an increased virulence of the amebae.

The observations of Meleney and Frye are of special importance as contributions toward the solving of the problem under consideration. In 1933, these observers studied five strains of *E. histolytica,* two from individuals in the hills of Tennessee, where there was 38 per cent of incidence with this parasite unaccompanied by much symptomatology, and three from a lowland district with only 19 per cent of incidence but where amebic dysentery and other symptoms were present. In these experiments, using cultures of the various strains, and 364 kittens, they found that the two strains from the hill people were less pathogenic than the three lowland strains. They demonstrated that with both hill and lowland strains obtained from symptomless carriers ulcerations were produced, although they were less pathogenic than strains from individuals with acute amebic dysentery and they concluded that "Strains of *E. histolytica* from different localities may show differences in the incidence of infection and in the amount of pathology produced in experimental animals." In a later paper (1933a), these investigators conclude from their experimental evidence that the removal of bacteria from cultures of *E. histolytica* greatly reduces the incidence of experimental infections

in kittens, but that bacteria alone are not the reasons for differences in pathogenicity between different strains, these depending upon differences in the pathogenic activity of the strains themselves. In a third contribution published in 1935, Meleney and Frye, continuing their studies upon the virulence of different strains of *E. histolytica* which had been cultivated for a period of nearly three years, 20 kittens being used in testing each strain, conclude that variations in virulence between the strains used are maintained for at least this period. They conclude from their experiments with the seven strains already mentioned that one strain from a symptomless carrier showed a very low degree of pathogenicity, and two other strains had an intermediate degree of pathogenicity, while four strains obtained from individuals infected during the Chicago epidemic showed a very high degree of pathogenicity. They consider that the large number of kittens used in the experiments are sufficient control of individual resistance to infection in the kittens and that by the use of a large number of animals they have proved that strains of *E. histolytica* of varying degrees of pathogenicity exist. From the standpoint of prophylaxis and treatment the concluding paragraph of this paper is of special interest. They say "Since even the least pathogenic strains which we have encountered produced lesions in some kittens, and since human beings may harbor potentially virulent strains without showing clinical symptoms, it is important that every person encountered in medical practice who is found to harbor *E. histolytica* should be treated with an amebicidal drug."

Faust and Swartzwelder (1935) found that the continuous passage of a strain of *E. histolytica* through experimental dogs resulted in a marked shortening of the period of incubation and in the extent and severity of both the macroscopical and microscopical lesions produced. These investigators based their conclusion on the successful passage of a single human strain of *E. histolytica* through a direct line of 20 dogs and subsidiary infection of 40 additional dogs. The original strain was obtained from a case of amebic dysentery contracted in Louisiana and the ameba belonged to a medium sized strain. In their experiments the influence of bacteria was controlled by culturing on beef broth the bacteria taken directly from the most fulminating cases and the inoculation intracecally of large amounts of the mixed cultures into negative animals. In none of the animals so inoculated was there any evidence of dysentery or diarrhea and at autopsy the large intestine of each animal was completely free of any inflammatory areas.

Meleney and Frye in a paper presented before the 1935 meeting of the American Society of Tropical Medicine and still unpublished, reported the results of continued experiments with two of the strains of *E. histolytica* that they had found to vary in virulence, using both dogs and

kittens in these experiments. Both strains had been maintained in culture for three years, one having a high pathogenic index and the other a low index. They found that in dogs and kittens *both* strains increased in virulence during repeated passage and that these strains when returned to culture tended to return to their original pathogenic index. In dogs inoculated with the strain having a high pathogenic index for kittens repeated direct passage resulted in a shortening of the prepatent period and the latter dogs exhibited more extensive and severe lesions. This strain upon being returned to culture and then used in kittens was found to have a higher pathogenic index than before passage through the dogs. The other strain, having a low pathogenic index, behaved in a similar manner, but was less pathogenic to dogs as it had previously been to kittens. These investigators were unable to show that bacteria had any effect in increasing the virulence and they conclude that usually bacteria do not sensitize the tissues to the amebae or cause lesions making a portal entry into the tissues for the amebae but the usually secondary invaders. Here again, these investigators have apparently shown that variations in virulence are fixed and characteristic of different strains of *E. histolytica.*

While the results obtained by the observers mentioned would appear to demonstrate that certain strains of *E. histolytica* are characterized by variations in virulence or that the virulence of strains may be raised by continuous passage through animals, results of other observers are not in agreement with them. Thus Werner (1908), Hartmann (1912) and Darling (1913) all believed from their experimental evidence that *E. histolytica* became *less* virulent after passage through kittens, while Dale and Dobell (1917) could detect no evidence of increased virulence in a strain which they carried through 46 passages in kittens. Still other investigators have apparently shown that variations in virulence noted in different strains are due to the bacteria present in the inoculum rather than to the virulence of the strains *per se.* Thus Cleveland and Sanders (1930), considered that the increase in virulence after passage of cultural *E. histolytica* through kittens was due to increased virulence in the bacteria present in the cultures and in a recent paper Spector (1935) found that the rectal injection of cultures of various bacteria with cultures of a strain of *E. histolytica* was followed by marked variations in the degree of amebic ulceration following infection. Thus, of 45 kittens inoculated with a strain of *E. histolytica* and hemolytic streptococcus, 43 developed dysentery, 41 showing severe ulceration and two mild ulceration at autopsy; of 23 kittens inoculated with *E. histolytica* and cultures of a green streptococcus, 20 developed dysentery and showed varying degrees of ulceration; of 56 kittens inoculated with *E. histolytica* and cultures of *Staphylococcus aureus* only one developed dysentery and this one had

severe ulcerations; of 19 kittens inoculated with *E. histolytica* and *Bacillus subtilis,* 10 developed dysentery, but of these five also showed a complicating streptococcus infection. None of the control kittens inoculated with the cultures alone showed ulceration and the author concludes that various species of bacteria produced marked variations in the virulence of different strains of *E. histolytica* and in the amount of pathology produced after the inoculation of such strains and bacteria.

I believe that it must be evident to anyone who reviews carefully the evidence now available regarding natural variations in virulence in different strains of *E. histolytica* and the possibility of increasing the virulence of certain strains by passage through animals that the preponderance of evidence is greatly in favor of the two propositions but that it must be admitted that the question still remains unsolved. While the experiments of Meleney and Frye in dogs and kittens, using a large number of animals, apparently controls the factor of host resistance so far as *these* animals are concerned, it by no means solves the problem in the human being. There is no doubt that in certain regions infection with this parasite is much more frequently followed by severe symptoms, as dysentery, than in other regions where the infection may be almost as common. Thus, in certain parts of the South a very high incidence of infection with *E. histolytica* is present with comparatively little severe symptomatology, while in regions in the tropics with no greater incidence of infection severe symptomatology is common. This can be explained, of course, by differences in virulence of the strains occurring in these regions, but it can be just as well explained by differences in the natural resistance of the infected individual. It has always been observed that infections acquired in the tropics are greatly improved, so far as symptomatology is concerned, upon removal to a temperate climate even though no medical treatment be administered. This was especially noticeable during the Philippine Insurrection, when it was invariably observed that soldiers suffering from amebic dysentery contracted in the Philippines began to improve as soon as they were transported to the United States, whereas those remaining in the Islands frequently died of the infection. This striking effect of a change in climate can not be explained by any effect upon the virulence of the parasite itself and the only conclusion justified is that the resistance of the individual to the infection was increased by removal to such a climate. Again the observations of Kagy and Faust (1930), of Faust (1932), Faust and Kagy (1933) and Faust, Scott and Swartzwelder (1934) upon the effect of certain food substances on the clinical character and experimental lesions produced in amebic infections in dogs very strikingly illustrate the profound influence of certain substances upon the resistance of the infected animal. They found that raw liver and liver extract were distinctly beneficial to dogs suffering from

amebic dysentery, that ventriculin was consistently harmful, and that the animals that were resistant to the infection on a balanced diet could usually be infected when they were fed canned salmon. The oral administration of the latter was used routinely to exascerbate mild chronic or negative infections but when the salmon was macerated and introduced intracecally improvement was noticeable in the host while on return to oral administration the infection promptly fulminated. Such experiments would appear to indicate that host resistance can be influenced by certain substances administered orally and indicated the extreme importance of more extended observations upon host resistance to this parasite.

I believe that the evidence is sufficient to demonstrate that there exist certain strains of *E. histolytica* which are naturally more virulent than other strains but that up to the present time no strain of this organism has been demonstrated to be non-pathogenic or capable of living in the lumen of the bowel of man for indefinite periods without producing lesions. I also believe that the evidence is sufficient to demonstrate that the virulence of certain strains of *E. histolytica* can be raised by continuous passage through kittens and dogs, but that it is unproven that such an increase in virulence occurs during passage through man. A more extended study of the host reaction to infection with *E. histolytica* is indicated and it is doubtful if the results obtained with experimental animals will solve the problem so far as human infection is concerned.

III. *Immunity in Infections with E. histolytica.* Despite the fact that there are features in the epidemiology of amebiasis that point toward both natural and acquired immunity in this infection, very little research work has been done on this phase of the subject. The only evidence of *natural immunity* that we possess is afforded by the epidemiology of the infection and what data are available are so scattered and so confusing that it is impossible to arrive at a conclusion regarding the existence of natural immunity. It is a well-known fact that in all regions where the incidence of amebic infection is high a considerable proportion of the inhabitants escape infection, although apparently as much exposed to it as those who are infected. Whether this freedom from infection is due to a natural immunity which prevents the parasite from living in the tissues of the intestine or whether it may be due to lack of actual exposure is an open question. However, where the incidence of the infection is very high it is impossible to believe that all individuals living in a locality have not, from time to time, been exposed to the infection and it is for this reason, therefore, that I believe there does exist in certain individuals a natural immunity to the ameba.

It is also a fact that some individuals recover spontaneously from the infection. I have observed several cases in which *E. histolytica* was present in the stools for varying lengths of time and finally disappeared with-

out any treatment whatever. Perhaps these cases are instances of natural immunity, the parasites failing to penetrate the intestine and after multiplying in the lumen of the bowel for varying periods of time finally disappearing. Such instances certainly do demonstrate that natural resistance may be so great in certain individuals that the infection disappears spontaneously.

As regards the question of *acquired immunity* we have some evidence showing that so-called immune bodies may be produced in the blood stream by inoculation with cultures of *E. histolytica* or with antigens made from such cultures. Before considering this phenomenon it may be stated that approximately 50 per cent of individuals harboring *E. histolytica* show no evident clinical symptoms of the infection, thus indicating that continued infection with this organism appears to confer a relative immunity in such individuals, so far as symptomatology is concerned. It has also been noted by numerous observers that natives living in the tropics where amebic dysentery is common and the incidence of *E. histolytica* is high, frequently show a low incidence of dysentery while strangers coming to the same locality suffer severely from amebic dysentery, thus indicating that the native population is at least partially immune to the same strains that produce severe symptoms and lesions in individuals who have not lived in the locality for any length of time. All of these data point toward an acquired immunity produced by continued infection or reinfection with the parasite.

While the data relating to the production of immune substances in the blood serum of infected animals or man are comparatively few, they are very suggestive. In 1924, Wagener demonstrated that precipitins can be demonstrated in the blood serum of kittens infected with *E. histolytica,* using an antigen prepared by extracting scrapings of the ulcerated intestine from infected cats. Previous to her work Izar, in 1914, using aqueous extracts of fecal material containing *E. histolytica* and liver abscess contents obtained from infected kittens as antigens demonstrated the presence of complement fixing bodies in the blood serum of five individuals and three kittens infected with this parasite. In 1921, Scalas, using an aqueous extract of mucous flakes from feces containing *E. histolytica* obtained positive results in complement fixation in cases of amebic dysentery. Owing to the crude nature of the antigens employed by Izar and Scalas the results were unsatisfactory, confirmation was not forthcoming, and further investigation was abandoned. In 1927, without knowledge of the work of Izar and Scalas, I demonstrated that the blood serum of individuals infected with this parasite contained complement fixing bodies when tested with antigens prepared by extracting cultures of *E. histolytica* in absolute alcohol. In later contributions (1928, 1933, 1934), I described the technic and results of a complement fixation test

devised for use in the diagnosis of this condition. Since these observations the occurrence of complement fixation in amebiasis has been confirmed by several observers. Spector (1932), using a technic practically identical with mine, obtained positive results in human infections; Menendez (1932) immunized rabbits by the injection of extracts of cultures of *E. histolytica* and demonstrated the presence of complement fixing bodies in the blood serum of these animals. Heathman (1932) was also successful in demonstrating complement fixing bodies in the blood serum of rabbits immunized by injections of extracts of cultures of *E. histolytica,* while Sherwood and Heathman (1932) obtained complement fixation in the blood serum of human cases of amebic dysentery using an antigen prepared by extracting cultures of the parasite. In 1934, Tsuchiya published a modification of the complement fixation test for amebiasis as did Weiss and Arnold (1934). These investigators again confirmed the existence of complement fixing bodies in human infections with *E. histolytica* and Weiss and Arnold demonstrated that complement fixation could be produced in rabbits by inoculating them with such antigens.

From these numerous observations it is evident that specific complement fixing bodies occur in the blood of animals and individuals infected with *E. histolytica* and can be produced in animals by the injection of antigens prepared by the extraction of cultures of this parasite. The fact that such bodies exist and can be produced experimentally, and that complement fixation occurs not only in individuals suffering from the severe symptom-complex known as amebic dysentery, but also in carriers without symptoms, and that in fact the reaction is stronger in such carriers than in those having active symptoms, demonstrates that there exists a reaction between the tissues of the body and the parasite, resulting in the production of these substances. What relation complement fixing bodies bear to immunity is at present unknown, for while precipitins, agglutinins and complement fixing bodies are called immune bodies we are still uncertain as to the exact relation they bear to immunity. I believe it is reasonable to conclude that these bodies indicate a reaction which in all probability leads to more or less immunity in the infected animal or individual, but so far as scientific data are concerned we have none that support this belief except those that are furnished in the epidemiology of the infection. No experimental work has been accomplished, so far as I know, which demonstrates that an animal may be rendered immune to amebic infection.

An interesting example of local tissue immunity to amebic infection is furnished by the liver. It is well known that this organ is very resistant, for if we consider the large number of individuals infected with *E. histolytica* and the very small percentage that ever develop amebic

abscess of the liver it is evident that the organ is usually immune to the infection. Instances are on record in which *E. histolytica* has been found in the liver capillaries in sections unaccompanied by any evidence of inflammation and it is impossible to believe that the organisms do not frequently reach the liver through the portal circulation, for in sections of the intestine showing very slight pathology the amebae are frequently observed within the blood vessels. The writer has observed two cases in which injury to the liver resulted in the prompt formation of an amebic abscess, thus indicating that the amebae were already localized in that organ but that it was so resistant to infection that no symptoms were produced until the resistance was lowered by injury. I believe that in a considerable proportion of infections with *E. histolytica* whether symptoms be present or not, *E. histolytica* is carried to the liver, and that were it not for the natural resistance of this organ amebic abscess would be a very frequent complication instead of a rare one.

It is evident from this brief discussion of the subject of immunity in amebiasis that we are without any very definite or conclusive data regarding the subject, and it is also evident that it demands further research and that the probability points toward the existence of both natural and acquired immunity in this infection.

The possibility of a natural immunity or an acquired immunity to local strains of *E. histolytica* has already been mentioned and should also receive attention. The recent work upon malaria showing beyond question that individuals may become immune to strains of the plasmodia endemic in the locality in which they live are very suggestive as explaining the relative immunity of natives living in a region where *E. histolytica* is endemic. It is not unreasonable to believe that such natives may have developed an immunity to the effects of a local strain of the ameba from repeated infections, while newcomers, having no time to develop such an immunity, develop definite and severe symptomatology.

The problems in the parasitology of amebiasis which have been discussed are only a few of those that still remain unsolved, but I believe that they constitute some of the most important. It is believed that all of them are susceptible to solution through experimental research and that such research would result in valuable information regarding this very important parasitic infection of man.

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THE PRODUCTION OF ARTIFICIAL IMMUNITY IN DOGS AGAINST *ECHINOCOCCUS GRANULOSUS*

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INTRODUCTION

During the past several years an increasing amount of literature has appeared in the field of helminth immunity. Although there is a great diversity of opinion among investigators considerable evidence has accumulated indicating that high degrees of immunity may be developed against many of these parasitic organisms. Nineteen years ago Fujinami and Sueyasu (1916) reported that the presence of *Schistosoma japonica* in the body brings about immunity against further infection. Working with a horse and calves they noted that the animals became immune following an infection of short duration. Recently Clapham (1933) has summarized the literature in the field of helminth immunity and makes the following significant statement. "In summarizing these results we are bound to notice how very much of the evidence is contradictory. This is probably due to the different standards and criteria that are adopted by individual workers. It is obvious also that much more accurate data are necessary before we are able to understand the basic principles underlying the subject."

The most convincing experimental work reported in this field during the past few years has been that of Miller and his associates Massie, Gardiner and Kerr (1930–34). These investigators immunized albino rats by injecting suspensions of dried, powdered material of *Taenia taeniaeformis* subcutaneously and intraperitoneally. Following the injections they attempted to infect their animals by placing onchospheres of the parasite directly into the stomach. In the normal course of events the onchospheres become active, bore through the intestinal wall, are filtered out in the liver capillaries and in this organ develop tissue cysts containing strobilate cysticerci. The experiments were controlled carefully throughout. Control (unimmunized) animals developed the typical liver cysts in large numbers while the immunized rats showed a degree of protection sufficient to completely or almost completely inhibit cysts from growing. Miller (1932) also observed that in cats the presence of the mature worms (*Taenia taeniaeformis*) in the intestine did not pro-

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tect the host against subsequent superinfection with *Cysticercus fasciolaris,* the larval stage of the worm. He therefore demonstrated that there was a lack of protection against the adult form which is an intestinal parasite while conclusively showing that the albino rat could be made immune to superinfection with the larval stage which is a tissue parasite. It should be mentioned that the lack of protection in cats was based on the presence of the adult form in the intestine and not the result of the use of antigen injections thereby attempting to produce a general tissue response. Miller (1932) has also shown that it is possible to transmit to offspring an immunity against infection with the same parasite in albino rats. Miller and Gardiner (1932) have also shown that a high degree of protection can be afforded by the use of immune sera from rats infected with *Cysticercus fasciolaris* or artificially immunized by powdered worm material.

The work of Miller and his associates suggested to us the possibility of determining whether immune reactions could be produced against *Echinococcus granulosus.* This parasite is commonly found in Syria. In a series of studies on over five hundred dogs collected from the streets of Beirut we have found from one-fifth to one-fourth of them infected with the adult intestinal form. An investigation of slaughter house material has revealed that sheep and cattle raised in the surrounding country are frequently infected with liver and lung hydatid cysts. Hydatid disease in the human is frequently seen so that the parasite is responsible for a genuine problem in this region.

Echinococcus granulosus has a life cycle somewhat similar to that of *Taeniae taeniaeformis.* The adult worm lives in the small intestine of the dog family where it is found attached to the mucosa. Onchospheres are passed in the stools and accidentally ingested in food, water or by other means, by cattle, sheep and human beings. The onchospheres bore through the stomach or intestinal wall, are filtered out in the liver capillaries and develop into hydatid cysts. Some of the onchospheres may pass through the portal system to be filtered out in the capillary beds of other organs where cysts may be formed. Infected meat containing hydatid cysts with viable scolices are ingested by members of the dog family with subsequent development of the scolices into the adult form in the intestine. Thus two entirely different problems confront the investigator in dealing with *Echinococcus granulosus.* One of these concerns the possibility of producing an immunity in the definitive host (dog) against the intestinal stage of the life cycle. The second problem concerns itself with possible immunity production in the intermediate host against the cyst or tissue stage of the life cycle.

Dévé (1927) attempted to immunize rabbits against *Echinococcus granulosus* with unsuccessful results. This investigator prepared sheep

hydatid fluid and sand by tyndallization and injected two rabbits subcutaneously with 2.0 cc. of the fluid containing 0.3 cc. of the sand. Injections were repeated eight times at weekly intervals. Four days after the last injection living hydatid sand was introduced subcutaneously and growing cysts were found when the animals were examined microscopically three months later. Since the usual means of infection is by way of the alimentary canal the direct injection of living hydatid sand subcutaneously may have overcome locally any protective general reaction resulting from the attempts to immunize.

Dévé (1933) has also reported experimental failure to produce immunity in white rats by means of specific serotherapy and the same author (1934) reported that in white rats and rabbits repeated subcutaneous injections of hydatid membrane material did not produce preventive or curative reaction against echinococcic infection.

Turner, Berberian and Dennis (1933) successfully immunized a series of dogs against *Echinococcus granulosus*. In the preliminary investigation only two control and eight immunized dogs were used. The antigens were prepared in two ways. Scolices and germinative membrane of *Echinococcus granulosus* were obtained from fresh hydatid cysts of sheep and cattle. The material was dried in an incubator at 37° C., powdered and stored in bottles. The second antigen was prepared by using scolices, germinative membrane and cuticular membrane from fertile and non-fertile hydatid cysts, prepared and stored in the same manner. Before using a phenolized 1 per cent saline suspension was prepared. Five injections of the antigen were given intramuscularly at intervals of three to five days beginning with 0.5 cc. and increasing to 1.0 cc. of the suspension. Six to fifteen days following the last antigen injection the animals were fed scolices from fresh, fertile mixed cysts. Scolex feedings were made by administering a suspension through a stomach tube. Control animals were fed at the same time with scolex suspension from the same source. Subsequently these animals were sacrificed and autopsied, the intestines being examined macroscopically and with lens for evidence of *Echinococcus granulosus*. The intestines of the control animals were lined with thousands of *Echinococcus granulosus* while the immunized animals had from no parasites up to a total of six in the intestine. Although this suggested the possibility of producing a marked degree of resistance to *Echinococcus granulosus* infection in dogs the number of animals used was too small to be considered as conclusive evidence.

The present paper deals with the experimental data collected from investigations on a much larger group of adult dogs and a group of young puppies that have been under observation for the past two and a half years. The material deals solely with an attempt to immunize the

definitive host against the intestinal stage of the life cycle of *Echinococcus granulosus*. A parallel study on the possible production of immunity against the tissue or cyst stage in the intermediate host is being made and will be reported later.

EXPERIMENTAL DATA

I. *Studies on Adult Dogs*

(a) *Preliminary Data.*

Nineteen control and sixty-one immunized animals were used in these experiments. These dogs were selected from a group of over two hundred animals delivered to the University Laboratories by the city dog catchers. Each animal was isolated for a period of at least five days and daily stool examinations made. Selections were based on the absence of *taeniae* eggs in the stools. Ova or *Echinococcus granulosus* closely resemble those of other segmented *taeniae* so that differentiation on this basis alone was very difficult. It was impossible to be absolutely certain that we were always selecting clean animals as occasionally observations at autopsy indicated that a few of the selected animals had been infected in spite of the efforts to select *teaniae free* dogs.

In attempting to select clean animals the authors (Turner, Berberian and Dennis, 1935) considered the possibility of assistance by means of the intra-dermal Casoni reaction. Since the intestinal stage and not the tissue stage of the life cycle was being investigated any selections based on intra-dermal reactions were found to be totally unreliable and the method was discarded. It was therefore necessary to assume that some of the selected adult animals were infested with the parasite in spite of careful stool examinations. In an attempt to eliminate this uncertainty an investigation was also made by using a series of twenty-six young puppies raised in the laboratory under conditions insuring uninfected animals. (See Section II.)

(b) *Preparation of Antigen.*

Four types of antigen were used in these experiments and different groups of animals subjected to immunization with them.

Antigen No. 1: Scolex antigen.—Viable scolices from fertile lung and liver cysts from sheep and cattle were gathered and dried in an incubator at 37° C. When thoroughly dry the material was pulverized in a mortar into a fine powder. This was stored in tightly stoppered bottles. Just prior to use a 1 per cent suspension in 0.5 per cent phenol was prepared.

Antigen No. 2: Antigen from scolices and membranes (fertile cysts.).—Viable scolices, together with germinative and cuticular membranes from fertile cysts were collected and dried in the incubator at

37° C. When thoroughly dry the antigen was pulverized into a fine powder and stored in this state in stoppered bottles. At the time of use a 1 per cent suspension was freshly prepared in 0.5 per cent phenolized saline.

Antigen No. 3: Antigen from sterile cysts.—The thin cuticular membrane from sterile cysts of sheep and cattle was dried in an incubator at 37° C., pulverized and stored. The suspension was prepared for use in the same way as the previous antigens.

Antigen No. 4: New scolex antigen.—In view of the fact that the early antigens were difficult to keep in proper suspension thereby making accurate dosage and administration difficult the following technic was developed for the antigen used in the later experiments. Scolices from fertile hydatid cysts of sheep and cattle were gathered, a drop of formaldehyde added to prevent bacterial growth and the material dried in an incubator at 37° C., pulverized in a mortar and stored in tightly stoppered bottles. Just before using the required quantity of antigen was weighed and a fine emulsion prepared by grinding the powder in a mortar with slow addition of saline. The suspension prepared in this way was free of coarse particles and was quite stable. Two and three per cent suspensions prepared in this way could be used thereby making heavier antigen doses much easier to administer.

(c) *Control animals.*

Nineteen dogs were used in the control group. The animals were of various size, age, breed and sex. Each animal was isolated for several days in order to collect and examine the stools before attempts to infect by feeding were initiated. On completion of this examination the animals were fed material collected from fertile cysts. All material used in feeding was freshly collected from the city slaughter house. Material collected from several cysts was mixed and examined carefully to insure the presence of fertile, active scolices. The number of scolices fed to each animal was estimated by microscopic counts of the scolices per cubic centimeter. The number of scolices in the feedings given to this group varied from 3,000 to 50,000.

Autopsies were made from 12 to 197 days following the feeding of infected material. The average period of time allowed between feeding and autopsy was 84 days. The entire intestine was removed immediately after the death of the animal. The intestines were then opened with scissors and the mucosa examined macroscopically and with lens throughout its entire course. Questionable material was scraped from the mucosa and examined under the microscope.

Eighteen (94.6 per cent) of the control group were found to have *Echinococcus granulosus* in the intestine. Thirteen of them (70.1 per

cent) were very heavily infected and showed thousands of the small worms attached to the mucosa. Three of the dogs were very lightly infected in spite of the fact that they had received very heavy feedings. One of these animals had only 1, another only 2 and the third approximately 100 adult *Echinococcus granulosus* in spite of heavy feedings. The animal with two *Echinococcus granulosus* in the intestine had been fed 50,000 scolices. Only one animal in this group had an entirely negative intestine. This animal was autopsied 97 days following a feeding of 3,000 scolices from mixed fertile cysts. Two other animals fed on the same date with material from the same source showed heavy infection.

Although 94.6 per cent of these animals were infected and 70.1 per cent heavily infected it is of importance to note that some of the controls showed a marked resistance to heavy feedings of infected material and were only lightly infected. One animal was apparently totally unable to contract an infection and presented a clean intestine.

(d) *Animals immunized with Antigen from cuticular membrane from sterile cysts.. (Antigen No. 3.)*

This investigation included a small group of six dogs injected with material from cuticular membrane from sterile cysts (Antigen No. 3). Preliminary isolation and stool examinations were performed as on the controls. All of these animals received five doses of antigen, the initial dose in each case being 1.0 cc. of the phenolized 1 per cent suspension. The final dose of antigen was 2.5 cc. Antigen injections were given every 4 to 8 days. Feedings of mixed freshly collected cyst material were made at intervals of from 21 to 97 days following the final antigen injection. The number of scolices in the feedings varied from 1,000 to 10,000. The animals were autopsied from 10 to 120 days following the feedings and the intestines subjected to searching examinations as conducted in the control group.

Three of the dogs in this group had intestines that were entirely negative (50 per cent). One of the three had been fed 10,000 scolices 31 days following the last dose of antigen and another received 1,000 scolices 54 days following the last antigen injection. The third one of these negative animals received 1,000 scolices 97 days after the last antigen injection. Two of the animals (44 per cent) had intestines that were heavily infected. One of these dogs had received 10,000 scolices 23 days following the last antigen injection. This animal, however, died 10 days following the feeding of the mixed cyst material. Adult forms of *Echinococcus granulosus* were present in the intestine and obviously this animal had been infected before being selected for the experiment. The other heavily infected aanimal received a feeding of 5,000 scolices 40 days following the last antigen and was autopsied 120 days later. Be-

tween 3,000 and 4,000 *Echinococcus granulosus* were present in the intestine. The remaining animal in this group was autopsied 119 days following a feeding of 10,000 scolices. The intestine contained approximately 100 *Echinococcus granulosus.*

Half of the animals in this group were entirely negative and one of the infected animals with only 100 worms present showed marked resistance as controls fed at the same time were all heavily infected. The antigen prepared from cuticular membrane of sterile cysts therefore was responsible for a protective reaction developing in some of the animals of this group.

(e) *Animals immunized with Antigen from scolices and membranes of fertile cysts. (Antigen No. 2.)*

Thirty-two animals were studied in this group. Eight of the animals received five injections, fourteen received four injections, eight were given three injections and two received only two injections of the antigen. The interval allowed between the last antigen administration and the feeding of fertile mixed cyst material varied from 6 to 122 days. Feedings contained from 1,000 to 49,000 scolices. The interval between feeding and autopsy varied from 12 to 198 days with an average of 73 days.

Seventeen (53.0 per cent) of the thirty-two animals in this group had *Echinococcus granulosus* in the intestine at autopsy. Seven (21.8 per cent) of these infected animals had very heavy infections with the parasites numbering in the thousands. Fifteen (47 per cent) of the group had intestines that were entirely negative. Eight of the seventeen infected animals had very few parasites in the intestine, the total counts for each varying from one to six organisms. Two of the positive animals had moderate infections with the total number of *Echinococcus granulosus* approximately 200 in each one.

It is of interest in this series that four of the eight animals receiving five doses of antigen were completely negative and the other four had intestines containing one, six, and three *Echinococcus granulosus* while the remaining animal was heavily infected. Five of the fourteen animals receiving four injections of antigen had heavy infections, five were entirely negative, one had approximately 200 *Echinococcus granulosus* and the remaining three had from one to two of the worms present. Six of the eight animals receiving three injections had intestines that were entirely negative. One of the two infected ones in this group had only 3 *Echinococcus granulosus* while the remaining animal had approximately 150 of the parasites present in the small intestine. Of the two animals receiving two injections of antigen, one was entirely negative and the other one very heavily infected. These observations are summarized in

TABLE 1.—*Comparison of the Number of Antigen Injections with the Incidence of Infection (Antigen (No. 2) from Scolices and Membranes of Fertile Cysts)*

Number of antigen injections	Number of animals	Percentage of heavy infections	Percentage of light infections	Percentage of negative intestines
5	8	12.5	50.0	37.5
4	14	35.7	28.5	35.7
3	8	...	25.0	75.0
2	2	50.0	...	50.0

table 1. Unfortunately more animals with only two injections should have been used for comparative purposes. No remarkable difference is seen between those receiving three or five injections.

(f) *Animals immunized with Antigen from scolex suspension material from fertile cysts. (Antigen No. 1.)*

Twenty-three dogs were used in this group. Immunization was performed with from one to five injections of phenolized 1 per cent suspension of scolex antigen prepared from fertile cysts. Feedings of from 1,000 to 20,000 scolices from fresh fertile mixed cysts were made from 4 to 238 days following the last injection of antigen. Autopsies were made from 24 to 131 days following the attempts at infection.

Ten (43.5 per cent) of the animals in this group had entirely negative intestines while thirteen (56.5 per cent) were infected. Of the three animals receiving five doses of antigen two were negative and the remaining one had only five *Echinococcus granulosus* in the intestine. Of the five animals receiving four injections two had negative intestines at autopsy. Three had intestines containing 10, 12, and 200 parasites. The animal with 200 *Echinococcus granulosus* in the intestine had received a very heavy feeding of scolices (10,000) at the same time that another immunized animal was fed with the same material. The second animal was entirely negative. Controls for this group were heavily infected. Three of the eight animals receiving 3 injections were negative, two were heavily infected and the remaining three were very lightly in-

TABLE 2.—*Comparison of the Number of Antigen Injections with the Incidence of Infection (Antigen (No. 1) from Scolices of Fertile Cysts)*

Number of antigen injections	Number of animals	Percentage of heavy infections	Percentage of light infections	Percentage of negative intestines
5	3	...	33.3	66.6
4	5	...	60.0	40.0
3	8	25.0	37.5	37.5
2	5	40.0	40.0	20.0

fected (75, 50, 10). Two of the five animals receiving two injections were heavily infected, one was negative, while one had 16 and the other 129 *Echinococcus granulosus* in the intestine. These results are summarized in table 2. It will be noted that the negative intestines are most numerous in those animals receiving four or five injections while the incidence of heavy infections increases as the number of antigen injections decreases. This leads to the speculation that an even higher degree of protection might be afforded by more numerous antigen injections than have been used in this experiment.

(g) *Summary of data on control and immunized adult dogs.*

The results of the observations in the control and immunized adult dogs is summarized in table 3. It has been possible to infect nearly all

TABLE 3.—*Summary of Observation on Control and Immunized Adult Dogs*

Number of animals	Type of antigen used	Number of intestines infected	Number of intestines negative	Number of heavy infections
19	Controls	18(94.6%)	1 (5.4%)	13(70.2%)
6	Sterile cyst antigen (No. 3)	3(50.0%)	3(50.0%)	2(33.3%)
32	Fertile cyst scolices and membrane (No. 2)	17(53.0%)	15(47.0%)	7(21.8%)
23	Fertile cyst scolex suspension (No. 1)	13(56.3%)	10(43.0%)	5(21.7%)

(94.6 per cent) of the control animals and to obtain very heavy infections in over 70 per cent of them. In the immunized group it has been possible to infect approximately one half of the animals. Depending upon the antigen used from 21.7 per cent to 33.3 per cent of the immunized animals developed heavy infections which when compared to the 70.2 per cent of the control group indicates a definite protective response. From 43.0 per cent to 50.0 per cent of the immunized animals were entirely negative as compared to the one animal (5.4 per cent) that was negative in the control group. Here again a high degree of immunological protection is indicated in the animals subjected to antigen treatment.

In general those animals receiving four and five injections of antigen demonstrated a higher degree of resistance than those receiving only two or three injections. Little can be said regarding the duration of the protective reaction at the present time. Attempts to infect animals as late as 238 days following the last antigen injection have resulted in only very light infection although 10,000 scolices were in the feeding. No appreciable difference was noted in the results obtained with the three antigens used on adult dogs. Table 3 indicates a slightly higher incidence of heavy infections in the animals treated with sterile cyst antigen

(Antigen No. 3) but the group treated in this way was small and a larger group might readjust these figures. The results at best indicate only a partial, and by no means a complete protective response. The authors are unable to explain satisfactorily why some control animals are only lightly infected, one even negative, while other controls treated in exactly the same manner are heavily infected. It is possible that as Miller (1933) suggests these animals may be the offspring of infected females. On the other hand there is evidence accumulating which indicates that the pH of gastric and intestinal juices may have a great deal to do with determining infections of this type (Berberian—Unpublished observations).

The authors have also been confronted with the dilemma of attempting to explain why some immunized animals become heavily infected while others treated in identical ways had negative intestines. There is, of course, a corollary in the human where patients immunized against specific disease may show marked differences in their defence response. Some may subsequently succumb to disease while others exposed in the same degree are able to throw off infection. The mechanism, whatever it be, remains one of the puzzles to be clarified in the future.

II. *Studies on Puppies*

As indicated previously the eggs of *Echinococcus granulosus* are so similar to those of other taeniae that it was impossible, when dealing with adult animals, to be absolutely certain that clean animals were always being used. In order to avoid this obvious fault it was felt desirable to obtain young puppies, isolate and raise them under conditions where *Echinococcus granulosus* infection would be impossible. Puppies are notoriously difficult animals to experiment with, especially if kept in groups, as infections of various kinds spread easily. Pneumonia and distemper were exceedingly troublesome in the earlier experiments and it was finally necessary to isolate each animal in order to avoid contacts.

During the past winter and spring a group of 10 control and 16 immunized puppies were under observation. These animals were obtained as young as possible, isolated immediately and in cases where they were too young for experimentation allowed to grow for several weeks. Antigen injections and feedings were commenced when they were from eight to twelve weeks of age. The animals represented a very heterogeneous group of breeds, sex and size.

Antigen No. 4 was used in all cases for the immunized puppies. It was much easier to give and to keep in stable suspension than the earlier antigen preparations. The puppies were divided into four groups depending on the amount of antigen given and the number of scolices fed.

Group I consisted of two control and four immunized animals. The controls were fed 5,300 scolices from fresh mixed fertile cysts. At autopsy one of the controls carried 200 *Echinococcus granulosus* in the intestine while the other was very heavily infected with over 2,000 parasites. The immunized puppies in group I received three injections of Antigen No. 4. The initial dose was 0.5 to 1.0 cc. of 1 per cent suspension and the final dose from 1.0 to 1.5 cc. of a 3 per cent suspension. One of these puppies received 10,000 scolices in its feeding 27 days after the first antigen injection and on autopsy 76 days later had only one adult *Echinococcus granulosus* in the intestine. The other three puppies received 5,300 scolices in their feeding. At autopsy one intestine contained 40, another 50 and the third 28 *Echinococcus granulosus*.

Group II consisted of two control and two immunized animals. The controls were fed 4,000 scolices and at autopsy one carried 50 *Echinococcus granulosus* while the other exhibited a negative intestine. The two immunized animals received two injections of antigen (1.0 cc., 1 per cent, and 1.0 cc., 3 per cent). Feedings consisted of 4,000 scolices from the same material as was fed to the controls. At autopsy one intestine was negative and one contained 26 *Echinococcus granulosus*. This small group sheds no light on the problem and it was felt that something was wrong with the scolex feeding material used.

Group III consisted of four controls and four immunized animals. All received 5,000 scolices in their feedings on the same day. The immunized animals received two doses of 3 per cent antigen suspension (0.5 cc. and 2.0 cc.). At autopsy all of the controls were infected, one with approximately 3,000 parasites. Three of the immunized animals had negative intestines at autopsy and one was heavily infected.

Group IV consisted of two control and six immunized animals. The controls were fed 10,000 scolices and at autopsy were both very heavily infected. One had at least 4,000 *Echinococcus granulosus* present and the other so many that it was impossible to make a fair estimate (6,000–8,000). The immunized animals received 0.5 to 2.0 cc. of 3 per cent suspension of antigen in the first dose and 2.0 to 3.0 cc. of the same strength in the second dose. One animal received only one injection of 2.0 cc. of the 3 per cent suspension. Four of these immunized animals received 10,000 scolices in their feeding. All intestines were positive but with one exception relatively lightly infected when compared to the controls. Two received only 3,000 scolices and both exhibited light infection.

In these puppy groups there was a definite difference between the controls and the immunized animals. The number of scolices present was usually higher in animals of the control group. Best protection was afforded in those animals receiving two and three injections. Four and five doses of antigen were not given to these animals and this has prob-

TABLE 4.—*Summary of Observations on Control and Immunized Puppies*

Groups	Animal number	Date and amount of antigen injected	Interval between 1st infection and feeding in days	Date of feeding	Number of scolices fed	Interval between feeding and autopsy in days	Date of autopsy	Number of scolices present
Immunized animals Group I	1	2/22/35 1 cc. 1% 2/28/35 1.5 cc. 1% 3/18/35 1.0 cc. 3%	27	3/21/35	10,000	76	6/ 5 /35	1
	2	2/22/35 1.0 cc. 1% 2/28/35 1.5 cc. 1% 3/18/35 1.0 cc. 3%	27	3/21/35	5,300	76	6/ 5 /35	40
	3	2/22/35 0.5 cc. 1% 2/28/35 1.0 cc. 1% 3/18/35 1.0 cc. 3%	27	3/21/25	5,300	9	3/30/35	50
	4	2/22/35 0.5 cc. 1% 2/28/35 1.0 cc. 1% 3/18/25 1.5 cc. 3%	27	3/21/25	5,300	12	4/ 2 /35	28
Control animals Group I	101	.....	..	3/21/35	5,300	22	4/12/35	200
	102	.....	..	3/21/35	5,300	4	3/25/35	2000+
Immunized animals Group II	5	2/22/35 0.5 cc. 1% 2/28/35 1.0 cc. 1% 3/18/35 1.0 cc. 3%	34	3/28/35	4,000	46	5/13/35	0
	6	2/28/35 1.0 cc. 1% 3/18/35 1.0 cc. 3%	34	3/28/35	4,000	5	4/ 2 /35	26
Control animals Group II	103	.....	..	3/28/35	4,000	31	4/28/35	50
	104	.....	..	3/28/35	4,000	69	6/ 5 /35	0
Immunized animals Group III	14	2/28/35 0.5 cc. 1% 3/18/35 1.0 cc. 3% 3/28/35 2.0 cc. 3%	35	4/4/35	5,000	24	4/28/35	0
	16	3/18/35 0.5 cc. 3% 3/28/35 2.0 cc. 3%	17	4/4/35	5,000	24	4/28/35	0
	17	3/18/35 0.5 cc. 3% 3/28/35 2.0 cc. 3%	17	4/4/35	5,000	24	4/28/35	0
	19	3/18/35 0.5 cc. 3% 3/28/35 2.0 cc. 3%	17	4/4/35	5,000	62	6/ 5 /35	2000

TABLE 4.—*Continued*

Groups	Animal number	Date and amount of antigen injected	Interval between 1st infection and feeding in days	Date of feeding	Number of scolices fed	Interval between feeding and autopsy in days	Date of autopsy	Number of scolices present
Control animals Group III	27	.....	..	4/4/35	5,000	18	4/22/35	8
	28	.....	..	4/4/35	5,000	7	4/11/35	3000
	29	.....	..	4/4/35	5,000	16	4/20/35	12
	30	.....	..	4/4/35	5,000	11	4/15/35	12
Immunized animals Group IV	22	3/18/35 0.5 cc. 3% 3/28/35 2.0 cc. 3%	73	5/30/35	10,000	30	6/29/35	450
	47	5/2/35 2.0 cc. 3% 5/9/35 3.0 cc. 3%	28	5/30/35	10,000	30	6/29/35	5000 or 6000
	48	5/2/35 2.0 cc. 3% 5/9/35 3.0 cc. 3%	28	5/30/35	10,000	12	6/11/35	1000
	49	5/2/35 2.0 cc. 3% 5/9/35 3.0 cc. 3%	28	5/30/35	3,000	9	6/ 8 /35	30
	50	5/2/35 2.0 cc. 3% 5/9/35 3.0 cc. 3%	28	5/30/35	10,000	30	6/29/35	800
	59	5/10/35 0.2 cc. 3%	27	6/6/35	3,000	9	6/15/35	480
Control animals Group IV	60	.....	..	5/30/35	10,000	16	6/15/35	4000+
	61	.....	..	5/30/35	10,000	5	6/ 4 /35	6000 – 8000

ably greatly lowered the incidence of completely negative intestines in the immunized groups as compared with the adult dogs. The results of the observations on puppies are summarized in table 4.

SUMMARY

Using 79 adult dogs and 26 puppies the authors have demonstrated the production of partial protective reactions against infection with *Echinococcus granulosus* following the use of antigen prepared from hydatid cyst material.

Animals treated with the antigen preparations showed a marked decrease in the incidence and degree of intestinal infection with *Echinococcus granulosus* as compared to the very high incidence of infection produced in the immunized controls.

Little, if any, significant difference was noted in the results obtained from antigen prepared in four different methods. Protective reactions were apparently greatest in the animals receiving the largest number of antigen injections.

These studies further support the recent observations of other investigators (Miller, *et al.*) in the field of helminth immunity. Although the protection afforded has been only partial the evidence indicates definitely that immune reactions of this type can be produced. It is also apparent that some dogs possess a defence mechanism against infection with *Echinococcus granulosus* the source of which we are at present unable to elucidate.

Further investigation in this field including studies of the tissue fluid reactions (agglutinins, precipitins, etc.) before and after immunization are needed and will be reported later. A parallel study of the possibilities of producing immune reactions in the intermediate host against the tissue of cyst stage of the life cycle of the parasite is being conducted.

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THE COURSE OF INFECTION OF *TRYPANOSOMA DUTTONI* IN NORMAL AND IN SPLENECTOMIZED AND BLOCKADED MICE*

WILLIAM H. TALIAFERRO AND YELENA PAVLINOVA

Various rodents under natural conditions harbor trypanosomes which in general produce no noticeable impairment of their hosts and which as far as have been studied bear a striking resemblance to *Trypanosoma lewisi* of the rat. These are generally considered separate species because of slight differences in morphology and marked specificity for their vertebrate hosts, but it seems not improbable that, as pointed out by Wenyon (1926), most if not all of them represent races of *T. lewisi* which have become adapted to particular hosts. Thus, the previous work on *T. duttoni* in the mouse, which is reviewed in Laveran and Mesnil (1912) and need not be repeated here, emphasizes the resemblance of *T. duttoni* to *T. lewisi.* Much of the work presented in this and in a subsequent paper is in accord with this general belief. On the other hand, some of it shows an unexpected divergence although this is probably quantitative rather than qualitative in character.

MATERIALS AND METHODS

The present strain of *T. duttoni* was first found in a house mouse, *Mus musculus,* captured by the Bureau of Plague Prevention[1] of San Juan, Porto Rico, in January, 1928, and since that time has been kept exclusively in white mice by intraperitoneal or intravenous sub-inoculations of blood diluted in saline. As far as can be ascertained the course of the infection has not changed since its isolation, except that the mean size of the "adult" forms appears to be 2 to 3 μ longer (*vide infra*).

In following the course of the infections and in studying the morphology of the trypanosome, daily blood films were made of each mouse, dried in the air, fixed in absolute methyl alcohol and stained with MacNeal's tetrachrome stain by the method of Gingrich (1932) or with Giemsa stain. The number counts were ascertained as ratios of trypanosomes (generally 50) to erythrocytes. By reference to the approximate erythrocyte count these were then calculated as thousands of trypanosomes per cmm. No attempt was made to follow the changes in erythrocyte counts so that the erythrocytes per cmm. especially in splenectomized and blockaded mice may have dropped considerably with

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[1] We are indebted to Dr. Arturo L. Carrión for supplying the mice from which this strain was isolated.

a proportionate decrease in absolute trypanosome count. This method, however, shows the number trends.

The general routine for "blockading" mice was to inject 0.5 cc. of 7 per cent Higgins India ink in saline intraperitoneally and 0.5 cc. intravenously daily for 6 days prior to infection. Occasionally at the 5th or 6th injection the tail was so discolored from the seepage of small quantities of ink from previous injections that intravenous injection was impossible. In such cases the mouse was given 1 cc. intraperitoneally. Splenectomy was performed on the day preceding the initiation of the blockading procedure. Intravenous injections were always made into one of the tail veins. The 2 rats were treated similarly except that 5 per cent ink and dosages of 1 cc. were used.

The occurrence and rate of reproduction of the trypanosomes was studied by the following two methods:[1] (1) by obtaining the coefficient of variation of valid daily samples, as outlined by W. H. and L. G. Taliaferro (1922), and (2) by examining the daily films for division stages, modified from the method described by Taliaferro, Cannon and Goodloe (1931). By the first method the coefficient of variation for total length (including the free flagellum) was ascertained. The mid lines of 25 to 100 (usually 50) trypanosomes encountered consecutively without selection were drawn and measured[2] at daily intervals throughout an infection (every other day during the later period of an infection) and the coefficient of variation of each sample computed by means of the usual formula. This constant expresses variability in terms of percentages of the mean size. For example, in *T. lewisi,* a coefficient of variation of 20 to 30 per cent indicates a population in which reproduction is at its height, whereas one of 3 per cent indicates a population of adults in which there is no reproduction. (For considerations of the validity and a fuller discussion of the method see W. H. and L. G. Taliaferro, 1922.)

By the second method similar samples of 25 to 200 trypanosomes (ordinarily 50) were observed at the same intervals as above. Among these were ascertained the proportions of (1) dividing, (2) short forms of 25 μ or less,[3] (3) abnormally broad or long forms, (4) intermediate

[1] These two methods have features in common with the methods Robertson (1931a and b) used in studying the reproductive cycle in some African strains of trypanosomes. She used the distribution of the sizes of non-dividing trypanosomes (as measured by the total length of 100 forms) in conjunction with the percentage of dividing forms.

[2] When forms had progressed so far in division that multiple flagella were present, the daughter cells were each measured and tabulated separately.

[3] The most perplexing forms to differentiate were those around 25 μ. These were drawn and measured before placing them. The selection of 25 μ as the upper limit for young forms was made arbitrarily, but only after the examination and drawing of many forms had made it seem the most suitable point of departure as to whether reproduction was occurring or not.

forms (*i.e.,* not belonging to any of the other classes), and (5) non-reproducing "adult" forms about 28 to 34 μ in length and 2.5 μ in width. Representatives of these 5 classes are given in the 5 rows of trypanosomes in figure 1—one class to a row in the above mentioned order. To indicate the extent of reproduction most concisely in the table and graphs, these five classes are treated as the percentage of dividing and short forms (classes 1 and 2 combined) among the total number examined since the presence of either or both classes shows that division has taken place, and since the percentage of such forms in the total sample represents roughly the amount of division that has taken place. Thus, 20 to 40 per cent indicates a population in which reproduction is at its height, whereas 0 per cent indicates a population of adults in which there is no reproduction. Valid results can be secured with smaller samples by this method than by getting the percentage of only those forms actually in division. (*Cf.* the percentages in table 1 of young and dividing forms combined and of dividing forms alone.) For convenience of nomenclature throughout this paper, the dividing and short forms will be referred to together as "division forms" (*viz.,* indicative of division).

The data in this paper furnish an interesting comparison of the two methods of measuring the rate of reproduction. We have found both valuable but neither without its drawbacks. In general they compare very favorably and show the same trends. Occasionally, an unusual amount of intense division resulting in a large proportion (40 to 50 per cent) of small forms more or less uniform in size will give a coefficient of variation of 20 per cent and a percentage of division forms of 40 to 50 per cent (Fig. 5, 7th day). Such a discrepancy is, however, only one of degree since both register the occurrence of active reproduction.

In presenting the following experiments the mice whose infections are actually described are numbered consecutively, but they form only a part of the mice needed and observed in performing these experiments.

NORMAL COURSE OF INFECTION IN THE MOUSE

Briefly, *T. duttoni,* when introduced into the mouse, goes through an initial period of reproduction during which it increases in numbers showing some dividing, young and large forms, as seen in figure 1, 4–8, 15–21, and 22–26, respectively. Gradually it stops reproducing, with only forms seen such as Fig. 1, 30 to 50, and subsequently it lives in the blood of the mouse for from one to several months as non-reproducing adults (Fig. 1, 41–50) with an occasional form seen such as Fig. 1, 33 to 40. A strikingly peculiar form occurs extremely rarely. It appears to be dividing, but is slenderer than usual, is acidophilic in staining instead of basophilic and the potential daughter cells are about the same

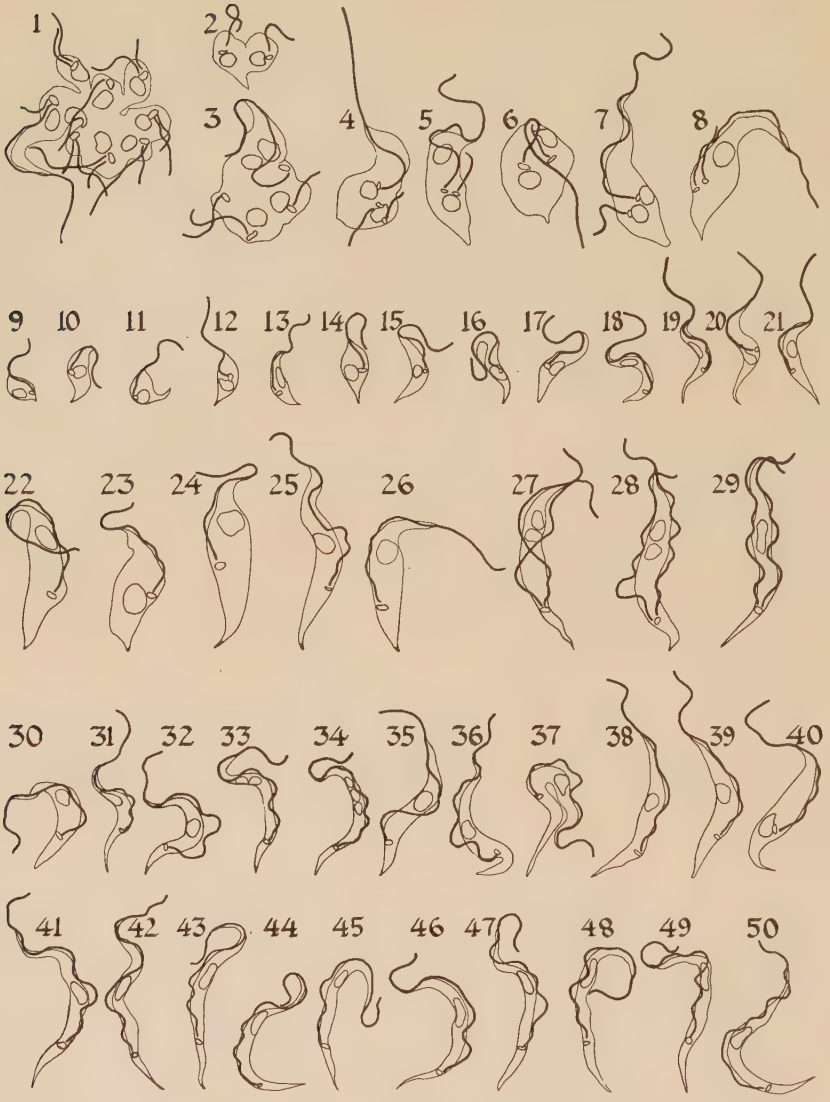

FIG. 1. Various stages of *T. duttoni* in the mouse: 1–8, dividing; 9–21, young forms, 22–26 large forms; 27–29 rare atypical large forms; 30–40 intermediate stages; 41–50, adults. All are found in splenectomized and blockaded mice, but only 5–8 and 15 to 50 are found in untreated mice (*i.e.*, normal infections). × 1000.

size (Fig. 1, 27–29). At first it was considered a dividing form, but its atypical aspect led us to consider it a double monster in which the component parts remained united instead of separating. Only about 10 or 12 of these forms have been seen, but have been found in splenectomized as well as in normal mice and during the "adult" period as well

as the reproductive period of the infection. Similar forms have been found in *T. lewisi* infections.

The outstanding difference between *T. lewisi* in the rat, as shown by Taliaferro (1924), and *T. duttoni* in the mouse is that ordinarily the reproductive period of *T. duttoni* is never as intense, but is more prolonged. In fact in many infections only diligent searching during a few days at the beginning of the infection revealed any frank division. This may readily be seen by comparing figure 2 showing an infection of *T. lewisi* in rat 265 and figure 3 showing an infection of *T. duttoni* in mouse 1. Each graph shows the rate of division (as expressed by the coefficient of variation and the percentage of division forms), the mean size in microns and the number curve.

The comparative inactivity of reproduction in *T. duttoni* is reflected in other ways. In *T. lewisi* (Fig. 2) the large proportion of young forms during the intense reproductive period just subsequent to the beginning of the infection significantly decreases the mean size as compared with the non-reproducing adults during the last part of the infection. Thus, the difference in mean size between the length of the trypanosomes on the 2nd day and at the time of inoculation was $4.63 \pm .54\ \mu$ and between the 2nd and 36th day was $3.89 \pm .50\ \mu$. In *T. duttoni,* on the other hand, there is never any striking difference and any difference

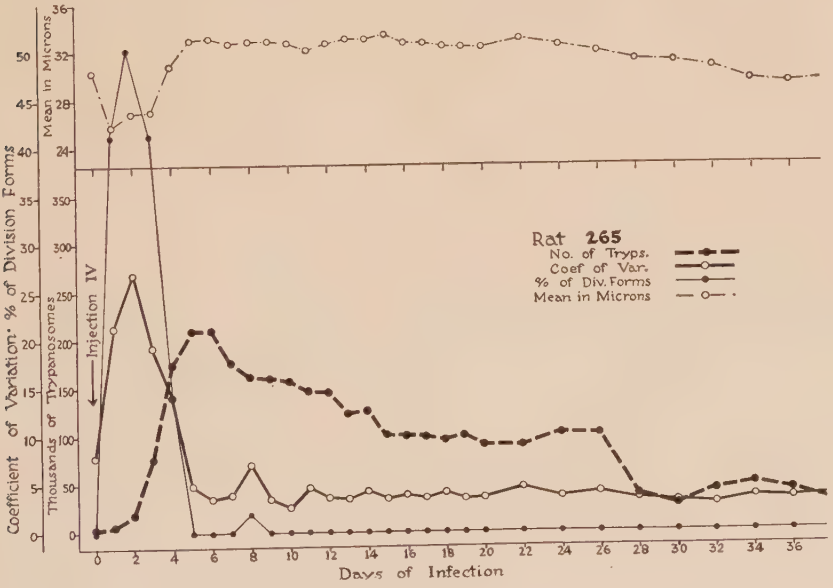

FIG. 2. Course of infection of *T. lewisi* in normal rat 265. Note the initial high reproductive activity and its abrupt cessation by about the fifth day (as shown by the curves of the coefficient of variation and the percentage of division forms,) and the concomitant increase in numbers (as shown by the number of trypanosomes per cmm.)

that occurs is not statistically significant. Furthermore, the retardation of reproduction in *T. duttoni* is not as uniform as in *T. lewisi.* Consequently, percentages in *T. duttoni* are uneven and occasional dividing or young forms may be sporadically found until the 40th day. Eventually, however, reproduction of the trypanosomes is entirely inhibited (in mouse 1, Fig. 3, after 18th day). This will be shown in a subsequent

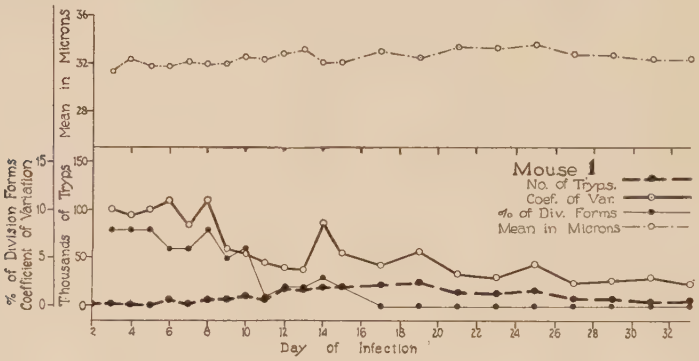

FIG. 3. Course of infection of *T. duttoni* in normal mouse 1. Note the sluggish but prolonged reproductive activity with its eventual cessation (Coefficient of variation and percentage of dividing forms) and the concomitant paucity in numbers (No. of trypanosomes).

paper to be due to the elaboration by the host of a reproduction-inhibiting antibody, which is similar to the one developed in the rat against *T. lewisi* (Taliaferro, 1924).

The number curves of the infections also reflect the difference in reproductive activity. Thus, in *T. lewisi* (Fig. 2) the number of trypanosomes increases rapidly during the initial period of reproduction until reproduction is inhibited. In *T. duttoni* (Fig. 3) the number of trypanosomes increases sluggishly during the initial period of reproduction and thereafter may increase slightly (since reproduction is not completely inhibited), but generally remains approximately stationary or slowly decreases until the infection is terminated.

Seven of forty *T. duttoni* infections gave out between 20 and 29 days, 22 between 30 and 50 days, 5 between 50 and 60 days, 5 equally distributed between 74 and 114 days, and 1 lasted 163 days. Thus, the length of the infection of *T. duttoni* is quite similar to that of *T. lewisi.*

The percentage of division forms was studied in 24 infections.[4] In all of these mice, division forms were never numerous (maximum was 14 per cent) and true dividing forms were scarce (4 per cent were occa-

[4] These mice were infected intravenously with sufficient trypanosomes so that they could be studied from the time of injection. When few trypanosomes were used for the infecting dose, the highest point of division occurred on the first day trypanosomes could be found in the blood.

sionally found). The mean percentages of division forms and of dividing forms alone from the first 15 days of infection in these mice may be found in columns 1 and 2 of table 1, respectively. They show a gradual increase and subsequent decrease with the peak in division forms occurring on the seventh day and of actually dividing forms on the fifth day. Sporadic young forms were encountered for as long as forty days, but only one dividing form was discovered from the fifteenth day to the end of these infections, and that one was found on the 19th day in one infection.

TABLE 1.—*The mean percentages*[1] *of young and dividing (Y + D) and of dividing forms alone (D) in random daily samples from 24 normal and 11 splenectomized and blockaded mice infected with T. duttoni and from 5 normal and 2 splenectomized and blockaded rats infected with T. lewisi*

Day of infection	Normal			Splenectomized and blockaded				
	Mice 2–25		Rats 265–269	Long-lived mice 41–48		Short-lived mice 49–52		Rats 270–271
	Columns:							
	1	2	3[2]	4	5	6	7	8[2]
	Y + D[3]	D[3]	Y + D	Y + D	D	Y + D	D	Y + D
1	4.0	.2	32	too few		too few		0
2	3.9	1.3	34	too few		27	4	1
3	6.7	.7	30	15	0	22	6	25
4	6.8	.9	17	19	6.2	15	6.7	59
5	8.5	1.6	5	19	2.0	31	6.9	22
6	8.6	.8	4	25	6.4	41	11.0	19
7	9.0	.5	1	25	3.7	22	1.9	31
8	6.7	.4	.2	12	1.0	31	5.5	3
9	4.0	.3	0	11	3.7	33	6.5	2
10	3.5	.1	0	9	1.3	37	8.3	3
11	2.4	.3	0	2	.4			1
12	1.8	.4	0	7	2.9			2
13	1.2	.2	0	5	0			1
14	.5	0	0	0	0			1
15	.7	0	0	.4	0			2

[1] To obtain each of these mean percentages 1000 forms were examined in columns 1 to 3; 200 to 300 in columns 4 and 5 and 150 in columns 6 to 8.

[2] The only times when dividing forms were found in the rats showed mean percentages as follows: In the 5 normal rats on the first, second and third day, there were .7, .3 and .8 per cent, respectively, and in the 2 splenectomized rats on the third, fourth, fifth, seventh, eighth, ninth, tenth, eleventh, twelfth, thirteenth, and fifteenth days, there were 1, .5, 1, 3, 2, 2, 2, 2, .6, .2, and 1 per cent, respectively. One of the splenectomized and blockaded rats died on the thirteenth and the other on the sixteenth day of the infection.

[3] Y + D = young and dividing, *i.e.*, division forms and D = dividing forms alone.

Incidentally, it should be said that even these percentages are high for normal infections, *i.e.*, infections where the mouse recovered. A few of these mice died during the period of observation, and division in these was more intense just before death. Thus, mouse 24 never showed any actually dividing forms until the 11th day of its infection, but on the 11th, 12th and 13th day (died on the 14th) it showed 6, 6 and 4 per cent, respectively. Two other mice (No. 22 and No. 23) which died on the 10th day of their infection, showed 1 to 4 per cent of actually dividing forms from the 2nd to the 5th day of their infections. Concomi-

tantly, the occurrence of young forms was increased to a proportionate degree. From these data, we may conclude that just as in *T. lewisi,* intercurrent infections, as well as splenectomy and blockade, to be discussed in the next section, are instrumental in lowering the functional level of the macrophage system.

Similar mean percentages from infections in 5 rats occur in column 3 and footnote 2 of table 1. Comparison of these with the mean percentages found in the mice corroborate the differences already described. Parenthetically, it may be noted that more actually dividing forms are encountered in the *T. duttoni* infections than in the *T. lewisi* infections (*Cf.* column 2 with footnote 2 in table 1).

The present work has been under way since the spring of 1928 and the question arises as to what changes may have resulted from frequent passage through the mouse without recourse to natural methods of insect transmission. The amount of reproduction and the general course of infection have not changed. There has, however, been a marked change in mean size. Thus, the means of the adult trypanosomes in mouse 1 which was infected on March 15, 1934, varied between 32 μ and 34 μ (Fig. 3), whereas the means of adult trypanosomes in 5 mice whose infections were studied and drawn during March, 1928, varied between 28 and 30.4 μ, differences which are statistically valid. These differences could not have arisen from technical inaccuracies as the measurements have been verified under identical conditions. It is interesting to note that both the original and the present mean sizes of *T. duttoni* fall within the limits of mean size for "wild" populations of *T. lewisi,* as found by Taliaferro (1926).

EFFECT OF SPLENECTOMY AND INDIA INK BLOCKADE ON THE COURSE OF INFECTION

Since the original work of Regendanz and Kikuth (1927), extensive experiments indicate that splenectomy and other factors, believed to lower the functional level of the macrophage system, profoundly alter the ordinary course of *T. lewisi* in the rat and in particular decrease or stop the formation of ablastin (*i.e.,* the reproduction-inhibiting antibody). These effects appear to be greatly accentuated in infections of *T. duttoni* in the mouse.

When mice are splenectomized and blockaded, as previously described, the resulting infection closely approaches that of *T. lewisi* in the *normal* rat. Thus, as may be seen in mouse 41, figure 4, the coefficient of variation rose to 35 per cent on the 6th day and division forms reached the same percentage. The mean size of the trypanosomes was significantly lower during the intense reproductive activity (3rd to 7th day and especially 6th and 7th day) and the number of trypanosomes

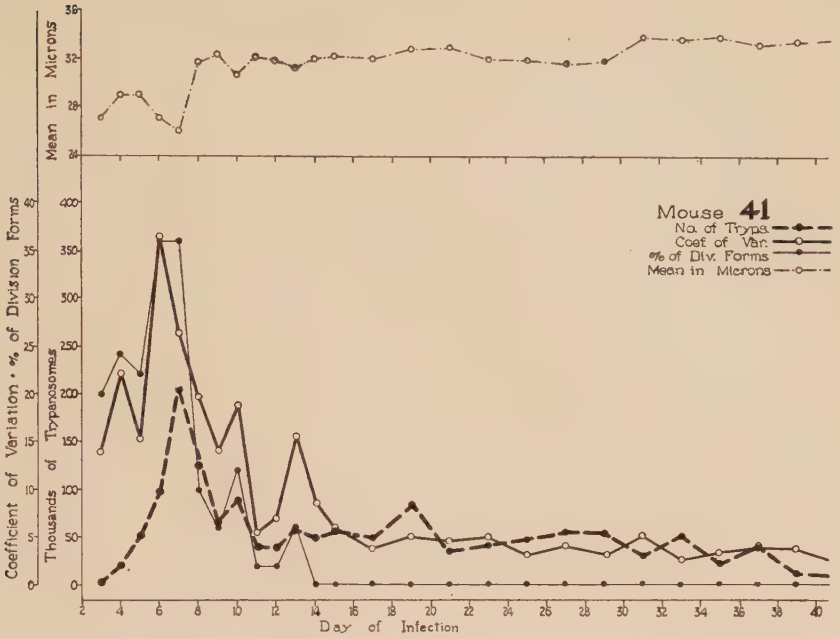

FIG. 4. Course of infection of *T. duttoni* in splenectomized and blockaded mouse 41. Note the initial high and prolonged reproductive activity with its eventual cessation (Coefficient of variation and percentage of dividing forms) and the concomitant increase in numbers (No. of trypanosomes).

reached 200,000 per cmm. Rosettes (Fig. 1, 1–3) and excessively small forms (Fig. 1, 9–14) made their appearance in this and similarly treated mice.

In some cases, intensive reproduction is prolonged as may be seen from the 4th to the 8th day of infection in mouse 42, Fig. 5. The number of trypanosomes in this mouse reached at the peak on the 6th day 1,450,000 per cmm. of blood which would be a very unusual infection even in *T. lewisi* in the rat. It is, however, interesting that reproduction is eventually inhibited in these experimentally altered mice—another evidence that splenectomy and blockade only temporarily lower macrophage activity.

Infections in 9 additional splenectomized and blockaded mice corroborated the findings just recorded. Both methods of determining the amount of reproduction were used on 6 of them and the inspection method on the other three. Mouse 43 most closely approximated the normal, but even it showed 20 per cent of division forms on the fifth day of its infection and for 8 days thereafter showed from 6 to 8 per cent. Three mice which died on the 10th day of their infection deviated most profoundly, and one of them showed from 30 to 58 per cent of division

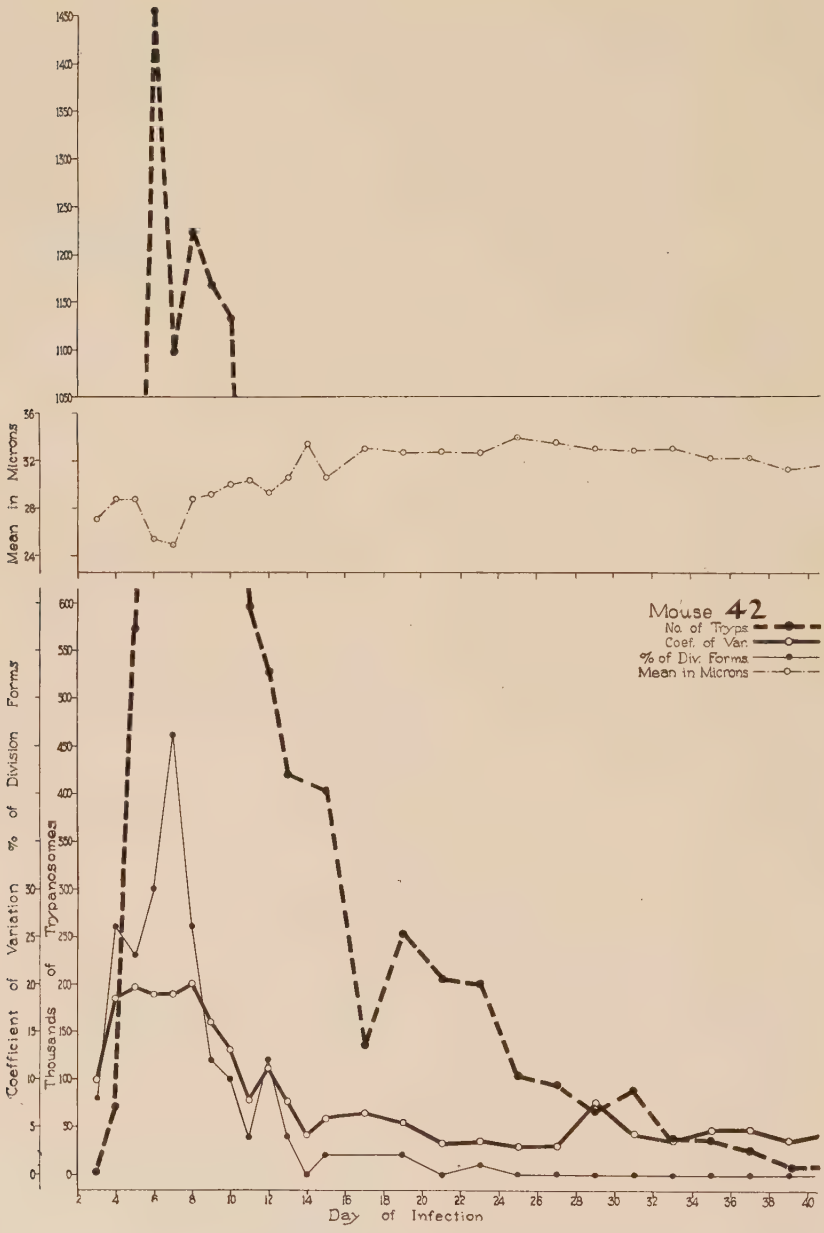

FIG. 5. Course of infection of *T. duttoni* in splenectomized and blockaded mouse 42. Note the prolonged heightened reproductive activity with its eventual cessation (Coefficient of variation and percentage of dividing forms) and the concomitant enormous increase in numbers (No. of trypanosomes).

forms consistently for 7 days. Mean percentages of division and of dividing (alone) forms have been arranged in table 1 in two groups from these mice; the first group from mice which survived the splenectomy, blockade and infection for more than 20 days (columns 4 and 5) and the second group from those which died between the 7th and 10th day of infection (columns 6 and 7). Inspection of these percentages and comparisons with the data from normal mice (Table 1, columns 1 and 2) show that reproduction of the trypanosomes is enhanced not only as regards its rate but also as regards its extent. *T. lewisi,* on the other hand, in splenectomized and blockaded rats (Table 1, column 8 and footnote 2) reproduced for a longer time, but not at a higher rate than in normal rats (Table 1, column 3 and footnote 2). The fact that a mean percentage of 59 per cent was reached on the third day of the infections in the 2 splenectomized and blockaded rats while only a maximum of 34 per cent was reached in the normal rats is only an apparent discrepancy and was due to the heterogeneity of the infections in the normal rats and the homogeneity of the infection in the operated rats. Thus, the infections in the two splenectomized and blockaded rats were derived from the same seed rat, equal numbers were injected, and the two rats were of the same age and died within 3 days of each other whereas the infections in the normal rats were derived from different seed rats, varying numbers were injected and the rats were assorted ages. In fact, maximum mean percentages from each of these normal rats equalled or exceeded 59 per cent at sometime (58, 66, 74 and 76 per cent, respectively, 22 to 50 hours[5] following infection). These data suggest that such procedures as splenectomy and blockade in the rat simply lessen the formation of ablastin (*i.e.,* acquired resistance) so that reproduction of *T. lewisi* lasts longer, whereas such procedures in the mouse lower a natural resistance, which normally prevents the trypanosomes from ever attaining their maximum rate of division, as well as depress the formation of ablastin (*i.e.,* acquired resistance).

DISCUSSION

The present study is of interest in demonstrating that the course of infection and the effects of host resistance in *T. duttoni* closely parallel the conditions in *T. lewisi* except that reproduction of *T. duttoni* is ordinarily never as pronounced as in *T. lewisi,* nor is it as quickly or as sharply inhibited. The course of infection with *T. duttoni* closely parallels the development of *T. iowensis* in the striped ground squirrel *Citellus tridecemlineatus,* as described by Roudabush and Becker (1934), and of *T. acouchii* in the agouti (unpublished work of the senior author).

[5] Blood smears were taken from these rats at hourly intervals.

Of particular interest is the fact that *T. duttoni* in splenectomized and blockaded mice is similar to *T. lewisi* in *normal* rats. This demonstrates that normal mice have a species or natural immunity which prevents reproduction of *T. duttoni* from the start of the infection and which presumably is associated with macrophage function, just as has been shown for *acquired* immunity by the rat to *T. lewisi.*

The fact that *T. duttoni* does not ordinarily reproduce as intensively as *T. lewisi* in the rat and the further fact that it does so after removal of the spleen and India ink blockade raises an interesting question in the evolution of the two parasites. On one hand, the greater ease of reproduction of *T. lewisi* suggests that it is better adapted to the rat than is *T. duttoni* to the mouse and that the latter may represent a strain of *T. lewisi* which has become partially adapted to the mouse. On the other hand, the assumption of many protozoologists that the longer the association of host and parasite the more the tendency to find the parasite harmless might suggest that the *T. duttoni*-mouse relationship is the older. As a consequence the mouse has developed a greater natural resistance to its parasite.

CONCLUSIONS

1. The normal course of infection of *T. duttoni* in the normal white mouse approximates that of *T. lewisi* in the normal white rat, but there are striking quantitative differences:

(a) Reproductive activity in *T. duttoni* is less and generally only a fraction of that observed in *T. lewisi* during the first of an infection, but continues at a minimal rate for several weeks.

(b) The number of trypanosomes is ordinarily much less than in infections of *T. lewisi.*

2. Both the reproductive activity of *T. duttoni* and the severity of resulting infections are greatly enhanced by splenectomy and India ink blockade carried out prior to infection. They are also adversely affected by intercurrent infections. Although part of this difference is due to a lowering of acquired resistance (ablastin formation), it is largely due to a lowering of the natural resistance of the mouse. This fact is of particular interest in showing that both natural and acquired resistance to the reproduction of the trypanosomes is dependent upon macrophage function.

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PHYLLODISTOMUM SINGULARE N. SP., A TREMATODE FROM THE URINARY BLADDER OF *DICAMPTODON ENSATUS* (ESCHSCHOLTZ), WITH NOTES ON RELATED SPECIES

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During the past several years the writer has examined twenty specimens of larval *Dicamptodon ensatus* (Eschscholtz) from Benton County, Oregon, for parasites. Four specimens of an apparently new species of *Phyllodistomum* were obtained from three of the salamanders. One specimen was embedded and sectioned serially, unfortunately without having first been studied and measured. The following description is based primarily on whole mounts of the remaining three specimens. The measurements given represent the average of the three specimens, with the range following in parentheses.

Phyllodistomum singulare n. sp.

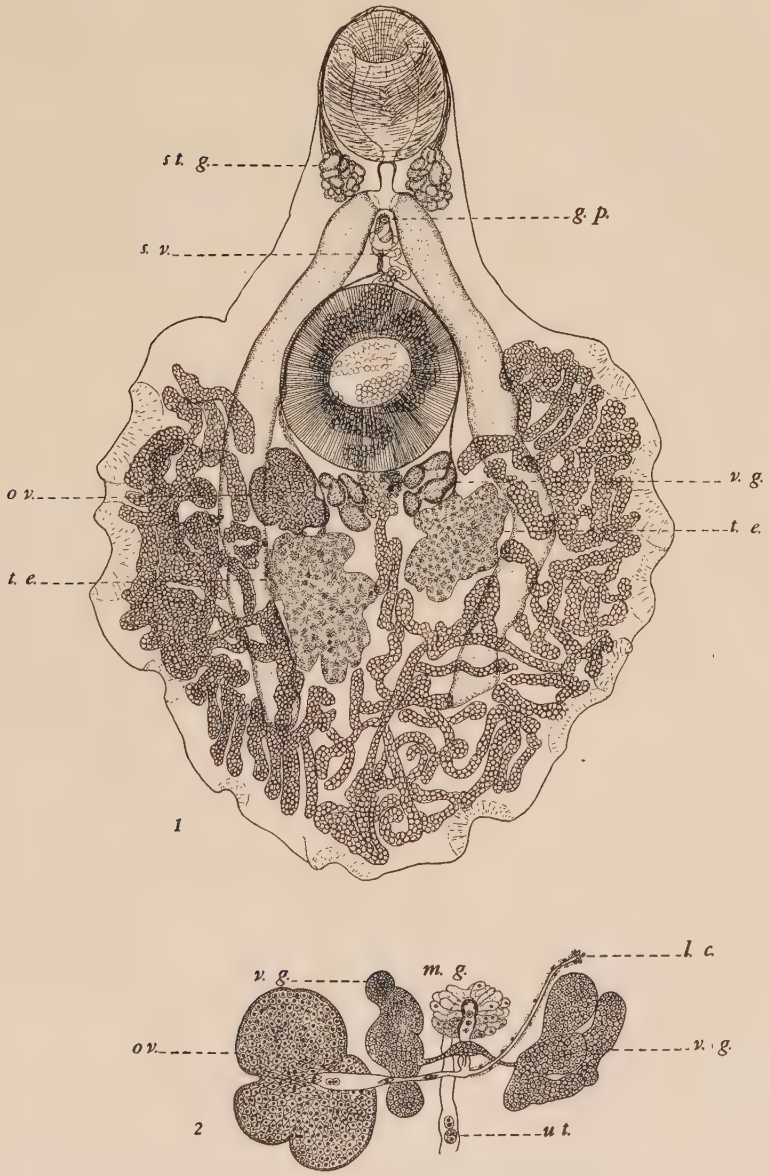
(Fig. 1, Pl. I)

Description.—Species with typical shape of genus, in particular of species sometimes referred to genus *Catoptroides*. Body composed of posterior discoid portion, rather sharply set off from narrow anterior part. Thin, flat, and unpigmented. In larger specimens margin of discoidal part noticeably crinkled; in smallest specimen this puckering of border not evident.

Length 3.6 (3.27–3.95) mm.; greatest breadth 2.77 (2.3–3.4) mm.; greatest thickness about 400 μ, except through acetabulum, which may protrude well beyond ventral surface; narrower anterior portion from 37 to 43 per cent of total length. Cuticle about 5 μ thick; in optical section border of cuticle appears finely serrate because of fine cuticular spines 1–3 μ in height. Mouth subterminal; oral sucker 675 (605–735)μ in longitudinal and 620 (600–645)μ in transverse diameter. Acetabulum 813 (650–905)μ in antero-posterior; 773 (665–835)μ in transverse diameter; its center slightly posterior to junction of anterior and posterior body regions. Muscular pharynx lacking. Oesophagus 166 (127–210)μ in length. Intestinal crura fail to reach posterior end by 18 (15–20) per cent of total length of worm.

Excretory vesicle slender, not visible in whole mounts; excretory pore in median line on dorsal surface at posterior end. Excretory vesicle originates between testes, just posterior to vitelline glands, by junction of right and left collecting tubes.

Longitudinal axes of all sex glands somewhat oblique. Testes irregularly lobed, near middle of posterior body region, one more or less diagonally in front of the other. Right testis 550 (385–740)μ by 337 (250–460)μ; left testis 513 (350–615)μ by 398 (380–435)μ. Vasa efferentia united just anterior to acetabulum to form short vas deferens which opens into seminal vesicle; seminal vesicle from 118 μ to 150 μ in length by 58 μ to 103 μ in width. Seminal vesicle terminates in narrower ductus ejaculatorius, the epithelium of which appears to be ciliated, although no ciliary movement detected in it in living worms. Seminal vesicle and ejaculatory duct dorsal to uterus; ejaculatory duct makes a U-shaped curve



EXPLANATION OF PLATE I

ABBREVIATIONS

g. p., genital pore; *l. c.*, Laurer's canal; *m. g.*, Mehlis's gland; *ov.*, ovary; *st. g.*, stylet glands; *s. v.*, seminal vesicle; *t. e.*, testis; *ut.*, uterus; *v. g.*, vitelline gland.

Fig. 1. *Phyllodistomum singulare* n. sp., ventral aspect. ×28.

Fig. 2. Diagram of female genital complex, reconstructed from serial sections and whole mounts. ×50.

to reach its external aperture just anterior to opening of metraterm. Because of its flexuous course, length of ejaculatory duct could not be determined. No cirrus or well-defined prostate gland. Genital pore from 225 μ to 300 μ anterior to acetabulum, close behind intestinal bifurcation, surrounded by rosette of radiating cells, possibly glandular.

Ovary either on right or left; in four specimens, on left in three, on right in one. Outline of ovary cut by shallow indentations into three or four broad lobes; longer axis of ovary 490 (460–505)μ; transverse diameter 358 (335–400)μ. Vitelline glands just behind acetabulum, on same level with ovary; each gland deeply and irregularly incised into from four to seven lobes. Right vitelline gland 382 (320–470)μ by 200 (175–250)μ; left vitelline gland 380 (340–410)μ by 180 (145–205)μ; junction of vitelline ducts may or may not be distended with yolk cells to form noticeable "yolk reservoir." Mehlis's gland between vitelline glands, poorly defined in whole mounts; Laurer's canal present; seminal receptacle absent.

Uterus exceedingly extensive, intricately coiled, and packed with eggs. Loops and coils of uterus occupy the extracaecal region posteriorly, and laterally to level of center of acetabulum. Central region between sex glands occupied by descending and ascending limbs of uterus; anterior to acetabulum uterus narrows into poorly defined metraterm. Metraterm with slightly more muscular wall than uterus, and in sections its epithelium appears to be ciliated. It continues anteriorly in winding course to open posteriorly to ductus ejaculatorius at genital pore; genital sinus so shallow as to be practically non-existent. Eggs within uterus of specimens mounted in balsam 32.5 (31–35)μ by 23 (22–24)μ, thin-shelled, non-operculate, containing embryo in advanced stage of development.

The most diagnostic character of the new species, by which it differs from all described species of the genus, is the presence of well-developed stylet glands in the sexually mature, and seemingly full-grown, worms. This feature is not unknown in the Gorgoderidae, since Looss (1902) reports similar "Kopfdrüsen" in adult *Plesiochorus cymbiformis*. The appearance of the stylet glands is that of a group of ten to fifteen large cells on each side of the oesophagus. The cells are of irregular and often vermiform shapes, and closely crowded, therefore their precise number could not be determined, but there probably are fewer cells present than there appear to be. Since a single group of cells forms a mass averaging 320 μ long by 187 μ wide, they present a far from inconspicuous character. The cytoplasm of the cells is finely granular and acidophil. The nuclei could only be found in sections; they are small, often angular in outline, from 6 μ to 10 μ in greatest diameter. From each group of cells several slender ducts pass anteriorly on each side of the oral sucker. They could be traced to the anterior end of the worm, although their precise terminations were obscure. They apparently open on the surface by several pores, anterior to and dorsal to the mouth.

Host.—*Dicamptodon ensatus* (Eschscholtz), larval stage.

Habitat.—Urinary bladder of host.

Locality.—Oliver Creek, Benton County, Oregon.

Type Specimens.—Two cotypes have been deposited with the United States National Museum.

Discussion.—The genus *Phyllodistomum* Braun, 1899 (syn. *Catoptroides* Odhner in Looss, 1902) has been reported from numerous freshwater and marine fish, and from caudate Amphibia. The genus *Catoptroides* Odhner was first diagnosed in 1902 by Looss, who utilized a name suggested to him by Odhner. The only differences between the genera, as defined by Looss, are that *Catoptroides* has the narrow anterior portion of the body sharply set off from the broad posterior part, and has the testes more nearly opposite each other. Such inconsequential

and variable features hardly constitute a valid basis for generic distinction. Indeed, the writer has whole mounts of *Ph. patellare* (Sturges), some of which agree with the diagnosis of *Catoptroides,* some with that of *Phyllodistomum,* depending on the state of extension when killed. The writer agrees with Nybelin (1926), who says: "Bei unserer gegenwärtigen, erweiterten Kenntnis der Phyllodistomen scheint es mir also kaum berechtigt, *Catoptroides* als selbständige, dem Genus *Phyllodistomum* ebenbürtige Gattung aufrecht zu erhalten."

The genus *Phyllodistomum* lacks a cirrus, the male ducts terminating simply in a conspicuous seminal vesicle. Holl (1929) mentions a cirrus sac in *Ph. carolini,* and again, in 1930, in *Ph. entercolpium.* Judging from his figures, Holl applied the term "cirrus sac" to the seminal vesicle.

MacCallum (1917) described and figured *Catoptroides magnum* as having a large, protrusible cirrus, and briefly mentions *C. aluterae* without either a figure or adequate description. Presumably both of his species have similar reproductive systems. The presence of a protrusible cirrus in these two species would seem to warrant their removal from the genus *Catoptroides* (=*Phyllodistomum*). They evidently are allied to the genus *Xystretrum* Linton, 1910, a Phyllodistomum-like Trematode with a protrusible cirrus. Travassos (1922) considers them to be species of his genus *Macia,* which as he points out, is closely similar to, but possibly distinct from, the genus *Xystretrum.*

The species of *Phyllodistomum* known from caudate Amphibia are *Ph. americanum* Osborn, 1903, from *Ambystoma tigrinum* Green and, from *Diemictylus pyrrhogaster* Boie, *Ph. patellare* (Sturges), 1897, and *Ph. entercolpium* Holl, 1930. *Ph. singulare* n. sp. can not be confused with *Ph. americanum* Osborn from which it differs markedly in every measurement and proportion except length.

The figures and measurements of *Ph. patellare* (Sturges) and *Ph. entercolpium* Holl are so nearly alike as to give rise to the suspicion that they are identical. The only important differences in the figures and descriptions of the authors are in the size of the testes, and in the eggs. Miss Sturges does not give measurements of the testes. The dimensions of the testes in the worm which she figures are estimated as 876 μ by 543 μ for the right; 981 μ by 557 μ for the left. Holl gives the measurements of the testes of his type specimen as 415 μ by 299 μ for the right; 429 μ by 415 μ for the left. In one of Holl's paratypes examined by the writer the right testis measured 530 μ by 624 μ; the left 600 μ by 520 μ. Sturges reports the eggs of *Ph. patellare* as measuring 25 μ by 17.5 μ; Holl gives the dimensions of the eggs of *Ph. entercolpium* as 39 μ by 25 μ.

Since *Diemictylus pyrrhogaster* is imported from Japan and offered for sale by dealers in aquarium-fish, the writer purchased twenty-six

salamanders from which thirty specimens of *Phyllodistomum* were obtained. Viable mature eggs are non-operculate, thin-shelled, and contain a fully-developed miracidium. They average 43 (38–46)μ by 27 (26–32) μ. In stained whole mounts the mature eggs had shrunken (usually without collapse or distortion) to 31 (27–37)μ by 21 (18–23)μ. The discrepancy in the egg-measurements of Sturges and of Holl is probably the result of variation in the extent of shrinkage resulting from the techniques employed, or possibly of differences in the maturity of the eggs measured. In the writer's preparations of *Ph. patellare* the range of size of the testes is from 505 μ by 200 μ to 1020 μ by 560 μ, which shows plainly that as regards the dimensions of the testes, Holl's specimens fall within the range of variation of *Ph. patellare.*

After carefully comparing the descriptions of Sturges and of Holl, examining a paratype specimen of *Ph. entercolpium* Holl kindly loaned by the U. S. National Museum, and studying numerous specimens of *Phyllodistomum* from the type host, the writer is of the opinion that the species *Ph. entercolpium* Holl is a synonym of *Ph. patellare* (Sturges), and therefore, by the Law of Priority, an invalid species.

Phyllodistomum singulare n. sp. differs from *Ph. patellare* (Sturges) which it closely resembles in dimensions and superficial appearance, in the following ways: *Ph. singulare* n. sp. retains the stylet glands to full maturity; its intestinal crura are shorter (the posterior 15 to 20 per cent of the body is free from intestine, as compared with 6 to 12 per cent in *Ph. patellare*); the walls of the intestinal crura are relatively smooth, whereas those of *Ph. patellare* are richly supplied with pronounced outpocketings; the vitelline glands are deeply incised into from four to seven lobes in contrast with those of *Ph. patellare* which are entire, or feebly lobed.

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COLPODA STEINI, A FACULTATIVE PARASITE OF THE LAND SLUG, *AGRIOLIMAX AGRESTIS*

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While examining land slugs for parasites during the autumn of 1933 numerous holotrichous ciliates were observed in the alimentary tract and other regions of the mollusc's body. The ciliate was identified as *Colpoda steini*. Since this had always been considered a free-living species a series of experiments were planned for the purpose of obtaining data relative to its proclivities as a facultative parasite.

THE ORGANISM

Maupas (1883) described *Colpoda steini* as a new species on the basis that it differed from *C. cucullus* O. F. M. in several respects. The most important differences are: *C. cucullus* 40–100 μ long, *C. steini* 25–45 μ long; *C. cucullus* 9–10 precytostomal lobes, *C. steini* 5–6 precytostomal lobes; *C. cucullus* macronucleus oblongate-oval, finely granular, without differentiation into a peripheral layer and central body; *C. steini* macronucleus spherical, composed of central core surrounded by a peripheral layer.

C. steini is widely distributed and has been reported occasionally from nearly all countries, though not so commonly as *C. cucullus*. It seems to be able to live wherever there is a little moisture and the ease with which it encysts enables it to withstand frequent and prolonged desiccation.

Ciliates may be found in freshly passed feces of infected snails—usually in the trophic stage. Of 107 recorded positive examinations only four showed encysted specimens, while all but one had motile forms.

Encystment will take place very quickly if the feces containing trophozoites are exposed to drying conditions. Apparently the length of time depends on the time required for the excess moisture in the feces to evaporate. This phenomenon has been observed to occur within one hour in a dry atmosphere; whereas, in a humid atmosphere trophic stages may be found in feces three days after being excreted.

The cysts produced in response to desiccation may be called "resistant" cysts in contradistinction to the "propagative" cysts from which they differ materially. These resistant cysts may be air dried and kept in a dry room for 12 months, after which they will give rise to motile forms within a few hours when placed in water. Furthermore, these cysts will resist prolonged (10 days) exposure to sub-freezing

temperature. On the other hand, the propagative cysts are formed in a liquid medium and represent a normal stage in reproduction.

The dimensions for *C. steini* given by Maupas are recorded in Table 1 for comparison with those of specimens used in this study. It will be noted that the ciliates from cultures (nearer the free-living condition) are smallest and those in the tissues are largest. The width of the *C. steini* in this study is greater in proportion to the length than Maupas found in his specimens. This may be accounted for by the fact that these measurements were taken from fixed specimens. In all other respects agreement between the two forms is very close.

TABLE 1.—*Maxima, minima and average dimensions of Colpoda steini as given by Maupas and for specimens in this study taken from cultures, feces and tissues. Our figures are based on measurements of fifty individuals selected at random*

Source		Maximum	Minimum	Aaverage
Maupas' figures	width	25 μ	9 μ	?
	length	45 μ	25 μ	?
Specimens from cultures	width	24 μ	14 μ	17.1 μ
	length	34 μ	19 μ	25.4 μ
Specimens from feces	width	44 μ	16 μ	30.3 μ
	length	59 μ	28 μ	41.2 μ
Specimens from tissues	width	38 μ	22 μ	30.5 μ
	length	58 μ	33 μ	44.6 μ

In species of *Colpoda* reproduction normally takes place with encystment followed by division into four daughter cells. Frequently only two cells are produced and occasionally eight are formed. If binary fission without encystment occurs in free-living specimens it is extremely rare. In spring water or in culture media *C. steini* exhibits the characteristic type of reproduction. In the tissues, however, the usual type of reproduction is by fission without the formation of cysts. In the snail's respiratory chamber, where the ciliates seem to thrive best, no sign of encystment has ever been observed—though frequent examples of binary fission are encountered.

EXPERIMENTAL

(1) *Snails Infected in Nature*

At first snails were killed and their alimentary tract opened. Later it was found that fecal examination yielded just as satisfactory results. In freshly passed feces trophic stages were easily recognized. If, however, the feces had dried examinations were facilitated by placing the material in a depression slide and adding water, allowing it to stand for twenty hours. By that time the ciliates, if present, would be actively swimming about in the medium.

If a snail were infected it would generally be revealed by the first examination. Occasionally one, two, or even more, negative examinations were followed by positive findings.

Snails were obtained from two sources. A. a wooded ravine, on the University campus, which terminated in a swampy outlet. One hundred and forty snails were examined from this area and all but eight were infected, or 94+%. B. a wooded hillside 1000 yards north of region A. Twenty-four snails were examined from this area and only six were infected, or 25%.

(2) *Methods*

Single snails were kept in clean Petri dishes in subdued light. A few drops of water were placed in the dishes to provide a humid atmosphere. The principal food was fresh lettuce. Twelve infected snails were fixed and sectioned in order to ascertain what parts of the snails' body were invaded by the parasites. Fixation in Bouin's picro-formal and the use of dioxan instead of xylene before placing the tissues in paraffine gave the best results. After the tissues had hardened the rudimentary shell was removed to avoid trouble in sectioning. Mallory's triple stain, Ehrlich's hematoxylin and Heidenhain's hematoxylin were used in staining. All gave good results.

(3) *Distribution of Ciliates in Tissues*

Examination of the sectioned snails revealed the ciliates distributed widely through the tissues of the body. The regions of most frequent occurrence and greatest abundance were the respiratory chamber and the anterior and posterior ends of the alimentary tract. In several cases they were present in the albumin glands, in two cases they had invaded the ovo-testis, and in one case fixed shortly after the snail had died (apparently from the infection) they had invaded the spongy tissues in the wall of the body.

Owing to the great concentration of the ciliates in the respiratory chamber, attention should be called to the proximity of the anus and the pulmonary orifice in this species. At the time of defecation an excellent opportunity is afforded the ciliates to pass into the respiratory chamber. *Vice versa* it seems probable that after an infection is established in the respiratory chamber ciliates may pass out of the pulmonary orifice and wander over the adjoining surface. Thus it would be possible for them to migrate into the posterior end of the alimentary tract through the anus. The evidence favors the mouth as the portal of entry, as will be shown from the following experiments:

(4) *Infection Experiments, Same Species*

After prolonged observation and frequent examination had proved certain snails to be free from infection, nine such animals were placed

in sterile Petri dishes and kept without food for twelve hours. Then small pieces of lettuce were thickly smeared with fresh feces taken from infected snails and a piece placed in each dish. Some of the snails readily ate the contaminated lettuce, others merely nibbled around the edges. Results of these experiments may be summarized as follows:

3 snails passed ciliates in feces in 12 hrs.
2 snails passed ciliates in feces in 22 hrs.
2 snails passed ciliates in feces in 36 hrs.
2 snails failed to become infected.

At the end of 36 hours they had eaten little or none of the contaminated lettuce. These last two snails were transferred to clean dishes and offered bits of fresh lettuce which had been smeared with fresh feces. Contaminated food was accepted this time and both snails were passing ciliates in their feces after 16 hours.

The infected snails were kept in separate containers and examined daily for two weeks. Since the infection was maintained in every case it was considered evident that a permanent infection had been established, consequently the daily examinations were discontinued. The snails died from 26 to 32 days after becoming infected and in every case numerous ciliates were found in the dead bodies. Naturally infected snails lived from 10 to 30 days after being brought into the laboratory. Uninfected snails could be kept alive more than two months.

(5) *Infection Experiments, Different Species*

A number of thecate snails, *Hyalina indentata,* found in region "A" were examined for parasitic ciliates with negative results. One such specimen, which repeated daily examinations had proved to be uninfected, was placed in a large dish with two infected slugs. After ten days it had acquired an infection which it maintained for two months (until death).

DISCUSSION

De Leon, 1919, found a ciliate in the intestinal tract of fresh-water snails (*Ampullaria*) in the Philippines. He described it as a new species of *Balantidium* (*B. haughwouti*) and stated that it was present in 99% of the snails. The heaviest infections were found in the mid and hind gut, being confined to the lumen. No evidence of tissue invasion was observed. When snails were kept in the laboratory for short periods there was a decrease in percentage of infection, and after a few weeks "examination of all snails will prove negative" (p. 393). The parasites showed effects of starvation when host was not well fed. It is obvious from these statements that *B. haughwouti* is not a true parasite. Apparently it feeds on the intestinal contents of its host

and is capable of withstanding the action of the digestive juices so long as the snail is in its natural environment. When, however, the host is placed under slightly different conditions, such as being brought into the laboratory, "the parasites apparently leave their host, either being discharged with the intestinal contents or swimming out by themselves to assume the exogenous phase in their life cycle" (p. 393). De Leon states further that it is not known if the ciliates divide outside of their host. They disappear from the water in four or five days, whether they encyst or succumb to toxic substances is unknown.

In several respects *Balantidium haughwouti* and *Colpoda steini* are similar. The morphological description and drawings given by de Leon indicate that he was dealing with a species of *Balantidium.*

It should be pointed out in this connection that the parasitic stage of *C. steini* resembles a *Balantidium* more closely than it does its own free-living stage. When one examines the stained nucleus, however, there is no need for confusion.

Most species of the genus *Balantidium* are parasitic; whereas most species of the genus *Colpoda* are free-living. That the ciliates involved in this study do not belong to a species other than *C. steini,* a species which is morphologically similar but physiologically different, is attested by the fact that they may be cultured *in vitro* with ease in almost any medium. They have been kept alive for two years in both a wheat medium and a medium containing Leibig's beef extract. Furthermore, morphologically identical forms have been cultured from the soil taken from the region in which the infected snails were found.

That the parasitic stage differs from the free-living stage is indicated not only by the fact that it thrives in a different environment but, as shown in Table 1, the size is considerably larger—and even more striking is the difference in the method of propagation. In cultures division always takes place within a cyst, while in the snail binary fission without encystment was the only type of division observed.

The ease with which snails may be infected in the laboratory by feeding them lettuce smeared with contaminated feces suggests the manner in which they acquire their infection in nature. *C. steini* is found in the moist soil of the snails' habitat. The snails live under leaves and stones, or even in loose soil. Furthermore they feed principally at night or in the early morning when dew is likely to be on vegetation. Under these conditions the ciliates might also be on the surface of vegetation and thus be ingested. A snail once infected would contaminate the leaves with its excrement containing motile forms, or cysts, and in this way most of the snail population would soon acquire the infection.

Snails kept, in the laboratory apparently succumbed to the infection in about one month. In nature the deleterious effects are probably not

so pronounced. The evidence indicates that even under natural conditions many snails are killed by the ciliates. The number of snails in region "A" where the infection percentage was 94+ has decreased approximately ½ during the past two years. The snails which have been removed for experimental purposes (about 300) could hardly account for the reduced numbers. Since these snails are frequently destructive to garden crops it is probable that the ciliates could be used to check the depredations.

SUMMARY

By way of conclusion the following points may be emphasized: 1. *Colpoda steini,* a free-living ciliate, readily adapts itself to living in various organs, especially the respiratory chamber, of the land slug, *Agriolimax agrestis.* 2. While in the snail host the ciliates become larger and change their morphological appearance and method of propagation. 3. Under laboratory conditions the ciliates produce a harmful effect on the snail host, accelerating if not causing death. A marked reduction in the number of snails from an infected area on the University of Virginia campus indicates that the ciliates may be useful in combating molluscan pests in nature.

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THE DEVELOPMENT OF TRICHINAE IN ABNORMAL ENVIRONMENTS[1]

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The factors which initiate the development of helminth parasites in their hosts are not well understood. The complexity of the intestinal and tissue environments in which worms develop makes it practically impossible to evaluate the influence of the various elements present. The limitation of most worm species to a restricted number of hosts suggests that small biochemical differences in the host environments must determine whether or not the parasite can develop. In spite of numerous attempts, no one has been able as yet to cultivate the parasitic stages of any of the helminth parasites of vertebrates in an artificial medium.

The present experiments deal with attempts to elucidate the factors which govern the development of *Trichinella spiralis*. This parasite is a favorable species for study for several reasons. (1) It is able to develop in a wide variety of hosts, which include most mammals, and under some circumstances certain species of birds. (2) The infective larvae grow to sexual maturity in the short period of 3 days. (3) Since the infective larval stage develops in the muscle tissue of the host, the intestines of the larvae do not contain bacteria, and consequently it is quite easy to obtain sterile larvae for inoculation into artificial media. Although, so far, all attempts to obtain development to the adult stage in a lifeless medium have been unsuccessful, it has been possible to rear normal sexually mature worms in such unnatural environments as chick embryos and the uteri of pregnant rats.

Trichina larvae for inoculation were obtained free from their cysts by digesting heavily infected rat muscle at 37° C. in a medium of 0.7 per cent pepsin and 0.5 per cent hydrochloric acid. The larvae were strained through a 60-per-inch wire screen and washed repeatedly to free them from débris. They were then suspended for 5 minutes in a solution of 1 : 2,000 merthiolate, buffered with 0.07 per cent borax, and washed again 4 or 5 times with sterile normal saline. This treatment invariably served to free the larvae from contaminating bacteria, and apparently did not injure them nor affect their ability to develop.

[1] This investigation was aided by a grant from the Rockefeller Foundation. The writer is indebted to Miss Helen M. Dedrick for helpful assistance during the experiments.

DEVELOPMENT IN CHICK EMBRYOS

Several unsuccessful attempts were made to obtain development of trichina larvae in cultures of the Maitland type, composed of minced 10-day chick embryos suspended in Tyrode's solution. Although most of the larvae remained alive for 4 or 5 days or longer, none of them showed any signs of development. However, when larvae were injected into living chick embryos, a small number developed to sexual maturity.

The technique used was very simple. The egg shell was sterilized with tincture of metaphen, a pin-point sized hole made, and approximately 1,000 sterile larvae suspended in 0.1 cc. of saline injected into the region of the developing embryo. Embryos varying in age from 6 to 15 days were used, but the most favorable results were obtained in those from 12 to 14 days old. In all, 50 embryos were inoculated, but only about 40 per cent of them survived. Examinations were made from 1 to 8 days after the inoculation and worms in various stages of development were found in about 60 per cent of the eggs in which the embryo still lived. Most of the negative cases can probably be explained by the failure of the injection to penetrate the inner embryonic membranes. When larvae were deliberately injected into the yolk sac, the ones which were subsequently recovered never showed any sign of development. Also, in embryos which had died soon after injection, the larvae, although sometimes still alive, had not started to mature. In one experiment several eggs were left at room temperature after being inoculated. The embryos, of course, soon died and the larvae, although alive at the end of 7 days, remained undeveloped.

In the embryos which survived the inoculation, the number of developing worms found was usually less than 5 per cent of the number of larvae injected. Only a small proportion of the remainder of the larvae were recovered. Some of these were dead, others were alive but undeveloped. Many worms were probably missed owing to the difficulties of making a thorough examination. Blood shed during the process of removing the embryo from the shell often interfered with the finding of the worms.

The sexually mature trichinae found were almost always recovered from inside the amniotic sac, but in several instances a few had apparently developed in the peritoneal cavity of the embryo. Although sexually mature, the size of the worms found in embryos opened 6 or 7 days after inoculation was noticeably smaller than that of worms from the intestines of rats. In one embryo examined on the seventh day, a female worm was found which had been fertilized and contained young larvae developing in the uterus. Although it was possible for trichina larvae to develop to sexual maturity in living chick embryos, the environment was evidently not as favorable as the normal one in the intestine of a susceptible animal.

DEVELOPMENT IN THE UTERI OF PREGNANT RATS

In view of the successful development of adult trichinae in chick embryos, it was decided to test the ability of larvae to develop in rat embryos. Laparotomy was performed on pregnant rats on about the twelfth to fourteenth day of gestation, and approximately 1,000 sterile larvae suspended in 0.1 cc. of saline were injected through the uterine wall into the amniotic sac of the embryos. Usually from 4 to 8 embryos were inoculated in each rat. The growth of the trichinae in rat embryos proved to be much better than in the chick embryos. Practically all worms found in examinations made after from 2 to 5 days showed the same degree of development and increase in size that would be expected if they had grown in their normal intestinal habitat. On the fifth day after inoculation, numerous female worms were found with young larvae developing in the uterus.

In spite of careful search, only a relatively small proportion, about 10 per cent, of the larvae injected were recovered as adult worms, and no trace was seen of the remaining number except in embryos which had died soon after the injection. In these dead embryos, the larvae were usually disintegrating and covered with phagocytes, or if alive were very much stunted. The failure to find a larger number of adult worms in the living embryos is probably explained by the escape of many of the larvae through the needle hole in the embryonic membranes and the subsequent loss of most of them from the uterus.

At first it was thought that the larvae had to be introduced into the amniotic sac of an embryo in order for them to develop. It was soon noticed, however, that after such an inoculation adult trichinae could also be found free in the lumen of the uterus. In later experiments, accordingly, instead of injecting larvae into the amniotic sacs, they were simply injected into the lumen of the pregnant uterus. Approximately the same percentage of adult worms was recovered after this procedure as when the larvae were introduced into the amniotic sac. Some of the worms were observed to be partially embedded in the uterine wall or adhering to the chorion of the embryos. The majority, however, were free in the lumen.

Approximately 2,000 sterile larvae were injected into each horn of the uteri of four non-pregnant female rats. These animals were killed and examined, 1, 2, 4 and 5 days respectively after the inoculation. In the rat killed after 1 day, numerous larvae were recovered alive, but they were covered with phagocytes and none of them showed any sign of development. In the rats killed at a later time, only dead and disintegrating larvae covered with phagocytes were found. None had started to develop. Occasionally in the inoculated pregnant rats an adult worm was found with a few adhering phagocytes. The majority, however,

were entirely free from phagocytes. Apparently conditions are such in the pregnant uterus that phagocytes do not attack the larvae, whereas in the uterus of non-pregnant rats, the larvae are killed in from 24 to 48 hours. Phagocytes also destroy trichina larvae when they are injected into the peritoneal cavity of rats (McCoy, 1935).

The death of inoculated embryos invariably prevented or arrested the development of the larvae. In some instances, the larvae remained alive without development, but usually they were dead. One pregnant rat in which 1,500 larvae had been injected into the lumen of each horn of the uterus, was killed and examined 3 days after inoculation. The right horn contained 11 embryos, only 5 of which were living. Of the 7 dead embryos, 6 appeared to have died within 24 hours. Considerable blood-tinged fluid had accumulated in the lumen of the uterus from which 43 normal living adult trichinae were recovered. In addition, 26 sexually mature female worms were found dead and partially covered with phagocytes. The death of these worms, which must have occurred within 24 hours, apparently coincided with the death of the embryos. All observations indicated that normal adult trichinae could only develop in the presence of living embryos.

Four inoculated pregnant rats were allowed to give birth to their litters. The young were born 7, 8, 9 and 10 days respectively after inoculation. Six weeks later the mother rats were killed and all 4 were found to contain encysted larvae in their muscles. The carcasses were completely digested and 5,800, 21,300, 28,000, and 20,000 larvae respectively were recovered. These observations seem to indicate that normal migration of young larvae had taken place from the female worms which developed in the uterus. This conclusion appears plausible in view of the fact that female trichinae containing developing larvae were found in the uterus of all experimental rats examined 5 days or longer after inoculation. It is possible, however, that the larvae found in the muscles of the mother rats may have resulted from an intestinal infection acquired by licking up viable larvae which had escaped from the vagina after the intrauterine inoculation. Suitable control experiments to test this possibility were not devised.

Only 2 of the 4 inoculated mother rats raised their young. In 1 of these 2 litters, 5 young were raised, but in the other only 1 young one survived. The carcasses of these 6 young rats were subsequently digested and found to be free of encysted larvae. Since only about half of the embryos in the pregnant mothers were injected with larvae, it is quite possible that the young rats which were reared had never been inoculated. In any event, infection of young rats from female trichinae developed in the uterus has not been demonstrated.

DEVELOPMENT OF TRICHINAE IN THE UTERI OF PREGNANT IMMUNE RATS

Other work (McCoy, 1931, and 1935) has shown that, after recovery from infection with *Trichinella spiralis,* rats possess a potent immunity against reinfection. This immunity is manifested by the failure of larvae given by mouth in a test infection to develop in the intestine. A very few worms may start to develop, but usually they are lost from the intestine before new invasion of the muscles can take place. The finding that adult trichinae could develop in the uteri of pregnant rats afforded an opportunity to test whether or not the immunity in the intestine extended to other parts of the body.

Seven female rats, which had recovered from a moderate infection with *Trichinella* 4 months previously, were successfully bred. On about the fourteenth day of gestation, approximately 2,000 larvae were injected into each horn of the uterus. On the same day a test dose of 2,000 larvae was administered by stomach tube to make sure that the shock of the operation had not broken down the intestinal immunity. All 7 rats were examined from 2 to 4 days after inoculation. In 5 of the 7 animals, less than 5 per cent of the larvae fed by mouth had started to develop, indicating that these rats possessed immunity in the intestine. In the uteri of all 7 rats, however, a few adult trichinae were found to have developed. The numbers were in general comparable to those found in the uteri of control pregnant rats. Owing to the small numbers of adult worms which developed in the uteri of both the normal and immune animals, it was not possible to make significant quantitative comparisons. The results which were obtained, however, indicated that immunity in the intestine did not extend to the uterus.

DISCUSSION

The foregoing experiments show that adult trichinae are able to develop normally in an environment quite different from their usual intestinal habitat. Although little progress has been made toward determining the actual factors that initiate the development of the worms, it can be stated that certain factors such as digestive enzymes, bacteria or their products, and the partially digested food materials usually present in the intestine are not essential for their growth. Also, the failure of trichinae to develop in the amniotic sacs of dead embryos indicates that living host tissue probably is necessary for their development. These findings support the observations of Heller (1933) who reported some ingenious experiments designed to prove that *Trichinella spiralis* must derive its food from the intestinal mucosa of the host. Muscle containing encysted trichina larvae was enclosed in collodion sacs, and the sacs introduced into the small intestine of susceptible hosts (cats and rats). The muscle

was digested in from 6 to 8 hours, indicating that the sacs were permeable to digestive juice. In examinations made after from 1 to 3 days, the larvae in the sacs, although still alive, did not show any sign of development. Also, larvae enclosed in fine silk bags and thus kept from contact with the intestinal mucosa, did not develop. From these and other experiments, Heller came to the conclusion that the parasite must depend upon intestinal tissue for nourishment.

The reason why trichinae developed in the lumen of the uteri of pregnant rats and not in the uteri of non-pregnant rats is not clear. The much richer blood supply of the pregnant uterus may be a factor, or possibly the presence of certain substances connected with embryonic growth. Attempts, however, to obtain development of larvae *in vitro* in sterile amniotic fluid from both chick and rat embryos were not successful. The absence of active phagocytosis of the larvae in pregnant uteri was probably an important factor in permitting their development.

SUMMARY

When sterile larvae of *Trichinella spiralis* were injected into the membranes of living chick embryos or into the amniotic sacs of rat embryos, a small percentage of them developed to the adult stage. The rate of growth in rat embryos was the same as in the normal intestinal environment, but in chick embryos it was somewhat retarded. Development of trichinae also occurred in the lumen of the uteri of pregnant rats, but when larvae were injected into non-pregnant uteri, they were rapidly killed. In a few animals it appeared that normal migration of young trichinae to the muscles of the mother rat took place from female worms which had developed in the uterus. A small series of female rats showing intestinal immunity to a second infection with Trichinella larvae given by mouth failed to manifest any significant immunity in the uterus.

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THE EFFECT OF DIET ON THE COCCIDIAN INFECTION OF THE RAT*

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There have been but two papers of any note on the effect of diet on the course of experimental coccidiosis. The first by Allen (1932) seemed to have shown that a high-protein and high-vitamin (A and B) diet conditions in the common fowl an *Eimeria tenella* infection with a relatively low oöcyst production, a lessened amount of hemorrhage of short duration, a low mortality rate, and retarded recovery presumably associated with continued oöcyst production. The second by Jones (1934) led her to the conclusion that in the common fowl (1) vitamin-A did not influence the course of *Eimeria tenella* infections, (2) high-protein diets maintained the weight of the fowls at a level nearer that of the controls than the low-protein diets, (3) birds on a high-protein diet developed immunity to reinfection more slowly than those on low-protein diets, and (4) oöcyst production was higher among birds on high-protein diets. As Jones states, her experience that the birds on the high-protein diet maintained excellent physical condition is in direct contrast to Allen's results. The present series of experiments were planned to determine further the effect of diet on a coccidian infection. The rat was used as the host, and *Eimeria miyairii* the parasite. No claim is made that results obtained from this study are comparable to what has been determined in the fowl.

The rats used in the experiments were raised in the laboratory from birth. They remained with the mothers until about a month of age. After separation from the mothers they were fed Steenbock's growing ration and fresh milk until they were put onto the special diets. Usually the average weight was about 125 grams at the commencement of the test diets. The pellets of the rats were examined at least twice a week during the growth period in order to insure freedom from infection.

VITAMIN-A DEFICIENT DIET

The diet used to bring about a condition of vitamin-A deficiency was as follows: vitamin-free casein, 18 pts.; yeast (Fleishman's irradiated), 8 pts.; starch, 65 pts.; corn oil (Mazola), 5 pts.; salt mixture, 4 pts. The normals or controls received the same diet except that

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5 parts of butter was substituted for the corn oil. The animals were kept on these diets until the growth curves showed distinctly that those receiving corn oil had reached a weight level where they ceased to make definite gains, and likewise showed signs of xerophthalmia. The first infection with 1500 oöcysts was made at this time, and on 3 or 4 succeeding days. The counts were made on the tenth and thirteenth days of the infections.

There were ten rats in the deficient series and nine in the controls or normals. The counts for the deficient series ranged from 58 to 128 millions; mean, 82.5 ± 4.78 millions.

The counts for the controls ranged from 30 to 150 millions; mean, 79 ± 9.3 millions. Although the number of rats on the experiment was small, it is evident that vitamin-A plays little or no part in the quantitative character of the coccidian infection of the rat.

VITAMIN B AND G DEFICIENT DIET

No attempt to distinguish between the infection in vitamin-B and vitamin-G deficient hosts has as yet been made. The following diet, deficient in both vitamins, was used to bring the animals down to the point where it was certain that they were well depleted: vitamin-free casein, 18 parts; starch, 73 parts; butter, 4 parts; cod liver oil, 1 part; salts, 4 parts. The controls received the same diet made up to 8.0% with Fleishman's powdered yeast.

There were 15 rats in each of the two series, but the experiment was planned so that there were three sets of animals run at different times—with the same number of deficients and normals in each set—so as to facilitate counting. The animals were on their respective diets 9 or 10 days before the first infection with 1500 oöcysts. On successive days thereafter the infective doses consisted of 1500, 1500 and 3000 oöcysts. Elimination of oöcysts commenced on the seventh or eighth days and continued for 6 or 7 days. The oöcyst counts were made on the tenth and thirteenth days. Reinfection of the rats in both series after the cessation of oöcyst elimination was in all cases unsuccessful.

The rats in both series were weighed at frequent intervals in order to determine whether vitamin depletion was effective. In the case of one group of 6 avitaminized rats, the first 9 days on the diet produced a reduction in mean weight from an initial 91 gm. to 85 gm., while the 6 controls increased in mean weight from an initial 89 gm. to 103 gm. A second group of 4 deficients during 9 days decreased in mean weight from 72 gm. to 55 gm., while the 4 controls increased in mean weight from 72 gm. to 80 gm. A third series of 5 deficients which averaged 55 gm. on the day they were put on the special diet gained 0.4 gm. each during the first 10 days, while the controls on the normal diet

gained in average weight from an initial 55 gm. to 68 gm. The deficients in all cases showed steadily declining weights thereafter, while the controls showed normal growth gains. Thus there is every reason to believe that the diets intended to produce vitamins B and G deficiency and normal growth, respectively, accomplished their purpose.

The total number of oöcysts elminated by the rats of the B and G deficient series ranged from 2 million to 91 million; mean number of oöcysts, 35.8 ± 4.5 million.

The total numbers of oöcysts eliminated by the rats on the normal diet ranged from 106 million to 275 million; mean number of oöcysts, 167.27 ± 7.15 million.

The mean yield for the normal rats was approximately 4.6 times that for the B and G deficient series. It is to be noted that the lowest count in the normal series was somewhat higher than the highest count in the deficient series. The difference of the means divided by the standard deviation of the difference of the means gave a value of around 10.5, while 2 would have been significant. Another striking fact noted was the high variability in the counts for the deficient series. The coefficient of variation for this series was approximately 72%; for the normal series, only 24.5%.

VITAMIN D DEFICIENT DIET

The diet used to produce indications of vitamin D deficiency was as follows: yellow corn meal, 76 parts; wheat gluten, 20 parts; $CaCO_3$, 3 parts; sodium chloride, 1 part. The normals or controls received the same made up to 3% with cod liver oil. The rats were put onto the diet at 25 days of age and left on it for 22 days. The ulna and radius taken from sample rats at the end of this period and subjected to the well-known silver line test showed definite indications of rickets in the deficient series and none in the control series.

There were 12 rats in the deficient series and 12 in the controls, but the experiment was conducted in two sets consisting of 6 deficients and 6 normals each. The infective doses consisted of 1500 oöcysts each day for 5 days beginning on the twenty-second day after commencing the special diets.

The deficient rats of the first set eliminated from 76 million to 347 million oöcysts each; mean, 241.16 million. The normals of the same set, from 126 million to 322 million each; mean, 194.83 million. The deficients of the second set eliminated from 55 million to 106 million oöcysts each; mean, 74.83 million. The normals of the second set, from 36 million to 98 million each; mean, 65.83 million. The data were subjected to analysis of variance according to Snedecor (1934, pp. 40–44). An "F-value" of 1.82 was obtained for the ratio of the mean

square between deficient and controls to the interaction remainder, while 4.35 was required for significance. Hence there was no difference between the two series not explainable by random sampling.

FOSTER AND CORT'S RATION GENERALLY DEFICIENT IN VITAMINS, PROTEINS AND SALTS

Foster and Cort (1932) found that the resistance of undernourished dogs to the nematode worm *Ancyclostoma caninum* was materially lessened. The condition of malnutrition was brought about by keeping the animals over considerable periods of time on the following diet which is generally deficient in vitamins, protein and salts: corn starch, 35%; dried ground peas, 35%; mazola oil, 29%; NaCl, 1%; and an abundant supply of water.

In order to test out the effects of such a deficient diet in coccidiosis, 13 rats averaging about 120 gm. each received nothing but this mixture for a period of 20 days. On the seventh, eighth and ninth days the rats on the special diet and 11 controls receiving Steenbock's ration were each given 3000 oöcysts. The total oöcyst counts for the deficient series ranged from 35 million to 694 million; mean, 334 million. The same for the controls ranged from 160 million to 860 million; mean, 407 million. It is evident without statistical analysis that the generally deficient diet did not lower the hosts' resistance to the multiplication of the parasites in its tissues, although the rats on the diet were in extremely poor condition at the end of the experiment. The malnutrition was due largely, however, to reluctance to eat the deficient ration.

HIGH-PROTEIN DIETS

In view of the work by Hegner and his school demonstrating the adverse effect of high-protein diets on the flagellate population of the digestive tract of rats, it was decided to test for similar effects of high-protein diet on the coccidian population. As a preliminary test, 12 rats were fed a diet consisting of 1 part casein and 1 part of Steenbock's growing ration. The infective doses consisted of 1500 oöcysts daily for five days, beginning the eleventh day on the diet. The high-protein rats eliminated 202 million and 117 million oöcysts, respectively, and the normals 213 million and 158 million. A second set of 5 on a high-protein diet consisting of 70% casein eliminated 122, 136, 130, 73 and 156 million oöcysts, respectively; mean, 123.4 million oöcysts. The control series of 5 rats eliminated 80, 199, 191, 156 and 304 million oöcysts, respectively, mean, 186 million. Although the difference between the means is 62.8 million, it would be but 33.1 million if it were not for the one item of 304 million in the controls. Although a more extensive series might show a significant limiting effect of the

high-casein effect on the coccidial population, it is evident that such an effect is not of extreme magnitude, and it is doubtful if it could ever be of any practical significance. This is certainly not to say, however, that a more extended series of experimental animals might not show a significant difference between the quantitative character of coccidial infections in rats on high-protein and normal diets.

MEAT

Nine rats were heavily infected with uniform doses of sporulated oöcysts. The same day the Steenbock's ration was taken away from five of them, and henceforth they were given as much calf's liver as they could eat. The remaining four continued on Steenbock's. The mean oöcyst counts for the liver series was 295.4 million oöcysts; for the controls, 298.8 million. It appears that the liver diet was without effect.

YEAST

It has become a practice among certain poultrymen to supplement the rations of their poultry flocks with yeast in the hope of controlling coccidiosis. We have carried out two sets of experiments to test the effect of yeast on the coccidian population in rat infections.

Eight rats were placed on the Steenbock's ration supplemented with Fleishman's irradiated yeast to the extent of 10%. Ten controls received only Steenbock's ration. The total numbers of oöcysts eliminated by the controls ranged from 44 million to 261 million; mean, 152.7 million. The recipients of the yeast eliminated from 89 million to 253 million oöcysts each; mean, 169.1 million. Thus it is evident without statistical analysis that the irradiated yeast was without limiting effect.

A second experiment was performed using Fleishman's non-irradiated yeast to the extent of 10 % in Steenbock's as the trial ration. The salt element in this ration was 4% Osborne and Mendel's mixture. The controls received the straight Steenbock's ration made up with the regular salt content. The oöcyst yields from the 9 animals on the test series ranged from 27 million to 112 million; mean, 59.3 million. The yields from the 9 controls ranged from 29 million to 72 million; mean, 45.7 million. This experiment checks with the previous one in showing lack of value of yeast in reducing the parasite population in murine coccidiosis.

DESICCATION OF TISSUE OF HOST

Three half-grown rats on the Steenbock's ration were given only a limited amount of water in order to reduce their weight. At first

each rat was given 10 cc. of water daily, but the amount was gradually reduced until it was found that 4 cc. was easily sufficient to support life. The weight of one animal fell from 157 gm. to 131 gm. in 22 days; a second, from 146 gm. to 113 gm.; and a third, from 130 gm. to 100 gm. On the eighth day the 3 desiccated rats and 3 controls receiving water *ad libitum* were infected with 1500 sporulated oöcysts. The desiccated rats eliminated 141 million, 68 million and 44 oöcysts, respectively; mean, 84.3 million. The controls eliminated 210 million, 45 million and 139 million, respectively; mean 131.3 million.

An inspection of the data shows that the effect of the desiccation upon the coccidian population was practically nil. It should be stated that the lack of water reduced materially the amount of food consumed by the rats, although the weight records of the food were not kept.

LOW-SALT DIET

On Feb. 25, 1935, 5 rats averaging 40.4 gm. were placed on the Steenbock's ration without the salt, calcium or milk elements. The 5 controls of about the same weight received the same diet except that the regular amount of calcium carbonate and sodium chloride were added. On April 1, 2, and 3, 1935, each rat was infected with 1500 sporulated oöcysts of *Eimeria miyairii*. The rats continued on their respective diets until the end of the experiment on April 14, 1935. The duration of the experiment was 48 days. At the finish the mean for each lot was approximately 123 gm. Thus the low-salt diet did not have any effect upon growth.

The low-salt series eliminated from 81 million to 174 million oöcysts each; mean, 121.4 millions. The normals eliminated from 104 million to 158 million each; mean, 130 million. It is evident that the lack of supplementary salt elements in the diet was ineffective in modifying the natural course of the infections.

HIGH-SALT DIET

Six rats were put on Steenbock's growing ration made up to 4% with the well-known Osborne and Mendel's salt mixture, while the controls received the Steenbock's without the usual NaCl and $CaCO_3$. Three days later they were infected with 1500 oöcysts, and on the next day with 3000 oöcysts. The mean oöcyst count for the recipients of the salt mixture was 115.5 million; for the controls, 126.4 million. It is likely that the infection was not influenced by the supplementary salts.

ALFALFA

In view of the fact that coccidiosis in feeder cattle is believed by some to be due to the alfalfa (although there seem to be no published

statements to this effect), a series of rats was given Steenbock's ration made up to 5% with finely ground alfalfa beginning about a week before infection, and the controls were given only Steenbock's. The experiment was conducted with 14 animals on the special ration and 12 on the normal. The results will be described only briefly: the recipients of alfalfa eliminated a mean of 96.3 million oöcysts, and the controls 94.5 million oöcysts. The alfalfa apparently had no effect.

TOBACCO DUST

Three rats were put on Steenbock's ration made up to 2% with tobacco dust which in turn was reputed to contain about 2% nicotine. The controls received Steenbock's. The counts for the tobacco-fed rats were 160 million, 132 million and 115 million, respectively. Those for the controls were quite similar; viz., 144 million, 114 million, and 158 million, respectively. It is evident that the tobacco dust was without effect.

CONCLUSIONS

1. A vitamin-A deficient ration did not exert any modifying influence on the course of experimental coccidiosis when *Eimeria miyairii* was the parasite and the white rat the host.

2. A vitamin-B and vitamin-G deficient ration produced a very significant lessening of the number of oöcysts eliminated during the period of immunization. (Further work is being undertaken in order to determine which vitamin was responsible for this result.)

3. More oöcysts were eliminated by rats on a vitamin-D deficient ration than by the controls, but the results are not statistically significant.

4. A diet generally deficient in vitamins, proteins, and salts did not lower the host's resistance to the coccidian infection.

5. A high casein diet seemed to exert a slight limiting effect on the development of the coccidian population, but further work will be necessary to definitely establish this indicated result as a fact.

6. A diet of liver only did not affect the experimental course of the infection.

7. Yeast and irradiated yeast supplements to a normal or growing ration did not limit oöcyst production.

8. Desiccation of the host's tissues by depriving it of water did not alter the course of the infection.

9. Low- and high-salt diets did not affect the quantitative character of the infection.

10. Alfalfa and tobacco supplements to the ration neither increased nor decreased the number of oöcysts eliminated.

11. Since the possibly two effective methods of modifying the course of the infection [vitamins-B and G deficiency and high casein(?)] were extreme measures, it is necessary to carry on further work to determine possible methods of applying them before their practical application is recommended.

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THE MORPHOLOGY OF THE AMPHISTOME CERCARIA, *C. POCONENSIS* WILLEY, 1930, FROM THE SNAIL, *HELISOMA ANTROSA*

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During the course of a survey in 1929 of the larval trematodes infecting snails of the species *Helisoma antrosa* from a small pond in the Pocono Mountains at Henryville, Pennsylvania, 16 snails, about 4 per cent of those examined, were found to be infected with a new amphistome cercaria. In an abstract (Willey, 1930) the name *Cercaria poconensis* was given to the larva.

The snails were collected in October and 6 were kept alive for life history studies until early in April. These specimens continued to give off cercariae throughout this period. Of the others, some were crushed and the larval forms studied alive, and from the remainder serial sections of the entire digestive gland were prepared and stained in iron haematoxylin and eosin. Stained whole mounts of rediae and cercariae in various stages of development were prepared. In September of the following year (1930), 475 snails of the same species were collected from the same pond and under the same conditions. None of them were infected with any amphistome cercaria, although the infection with distome larvae was about the same as the previous year. Numerous collections of *Helisoma antrosa* from that locality since that time have shown no recurrence of the amphistome infection.

THE REDIA

When fully mature, the redia (Fig. 2) is sausage-shaped and rounded at both ends. In smaller, immature rediae (Fig. 3), however, the posterior end is usually much narrower and tapers from about the middle to become pointed posteriorly, while the anterior end is rounded and blunt. Mature rediae, containing from one to eight cercariae, vary in length from 0.468 mm. to 0.986 mm. The average length of 10 living rediae containing from 4 to 6 cercariae was 0.664 mm. The mouth is terminal and opens into a well developed muscular pharynx which is almost spherical and varies from 32 μ to 40 μ in diameter in the larger individuals. A short narrow esophagus enlarges into a saccular intestine which in young rediae extends back for three-fourths or more of the length of the body, and in large individuals is only about one-fifth of the body length. The redia moves sluggishly when freed from the snail and in a watch glass usually remains in one place with only slight changes in body shape due to muscular contraction. No traces of locomotor appen-

dages could be found at any stage, although stained whole mounts, sections and living material were carefully examined for them because of their importance in classification.

The rediae contain from 1 to 8 cercariae arranged progressively according to size, the smallest being most posterior, and several undifferentiated germ balls consisting of only a small number of cells are usually found near the posterior end. The arrangement of the larger cercariae according to size is not due in *C. poconensis* to the shape of the redia, as was reported by Beaver (1929) for *Allassostoma parvum,* because the sides of the redia in *C. poconensis* are practically parallel. A birth pore opens ventrally a short distance anterior to the termination of the gut.

The excretory system consists of 6 flame cells on each side of the body. In different specimens, portions of a system of collecting tubules could be traced on either side to the excretory pores situated on the lateral margins of the redia at about the middle of the body and the complete system has been reconstructed from observations on numerous individuals. In very young rediae, only 3 flame cells could be found on each side. At this stage, a tubule extends forward from the excretory pore to end in a flame cell just behind the level of the beginning of the intestine. Another tubule proceeds posteriorly, gives off a branch with a flame cell a short distance behind the excretory pore, and continues posteriorly to a flame cell near the posterior end. Later, each flame cell with its collecting duct divides giving rise to 6 cells on each side with the formula $2[(2)+(2+2)]$. The flame cells in living material are very large and conspicuous, the length of the flame being 18 μ. In the sectioned material the flame cells could be readily distinguished and the flame then measured only 14 μ. All the snails examined showed very many rediae of all sizes, but no sporocysts or mother rediae were found.

THE CERCARIA

C. poconensis, like most amphistome cercariae, is large and when mature is heavily pigmented, appearing as a black sphere when seen macroscopically in the water. It swims vigorously and almost continuously with its tail curved ventrally and forward. Under a coverglass the shape of the body varies from pyriform to a condition in which the sides are almost parallel with a gradual narrowing toward both ends. Living cercariae, after normal emergence from the snail, vary from 0.64 mm. to 0.98 mm. in length when measured under a No. 0 coverglass, but when under pressure of a No. 2 coverglass and with very little water, they flatten out and elongate to as much as 1.2 mm. The length of the larger cercariae found in the sections of infected snails measured between 0.4 mm. and 0.5 mm., but these were much contracted and somewhat rounded in shape due to contraction during the slow penetra-

tion of the fixing fluid through the body of the snail. The width of the living individuals varied between 0.455 mm. and 0.310 mm. Actual size of cercariae of a given species is probably much more uniform than such measurements indicate, since much depends on the weight of the glass and the amount of fluid under the coverglass.

The body wall contains a thick layer of dark brown pigment consisting of diffuse granules, and the structure of the cercaria is further obscured by elliptical cystogenous granules which fill almost all the available space in the dorsal half of the body whereas only a few scattered granules appear ventrally. They stain a deep blue with iron haematoxylin. Two large conical eyespots lie in the antero-posterior direction at a level between the buccal pouches and the intestinal bifurcation. The lenses are directed forward. The acetabulum is spherical and its diameter in 10 living specimens under a coverglass is 0.210 mm. The opening of the sucker is subterminal but under a coverglass becomes ventral in position. There is a rounded oral sucker, averaging 64 μ in width, from which two large buccal pouches extend backward and somewhat dorsally. A rather wide esophagus extends back a short distance from the sucker and opens into the intestinal caeca. Just in front of the bifurcation there is a very weakly developed esophageal sphincter or pharynx. The intestinal caeca extend laterally and backward and end blindly about 50 μ from the level of the anterior border of the acetabulum. The tail arises dorsally above the acetabulum and is contractile. When extended it is almost twice as long as the body and shows longitudinal striations.

The anlage of the genital system (Fig. 4) is well developed and appears at a very early stage. At the level of the anterior margin of the excretory bladder, a group of heavily staining cells constitute the rudiment of the ovary and accessory structures. A column of cells, two or three cells in width arises from this group and proceeds forward in about the median line to end in another large mass of cells lying ventral to the intestinal bifurcation. This mass represents the anlage of the structures associated with the genital orifice. Two additional groups of cells, the rudiments of the testes, lie near the median line at levels approximately two-thirds and one-third of the distance from ovary to the bifurcation. A single row of cells arises from each testis rudiment and join anteriorly

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EXPLANATION OF PLATE

Cercaria poconensis

Figures 1, 2 and 3 were made from free-hand drawings of living material.
Figure 4 drawn from stained whole mount using camera lucida.

Abbreviations: *bp*, buccal pouch; *eb*, excretory bladder; *ed*, excretory duct of tail; *ep*, excretory pore; *fl*, flame cell; *gb*, germ ball; *gm*, anterior genital mass; *ic*, immature cercaria; *ld*, lateral diverticulum of excretory duct; *ov*, female genital mass; *pd*, primary excretory duct; *pr*, remnant of pigment; *sd*, secondary excretory duct; *t*, testis rudiment; *vi*, vitellaria.

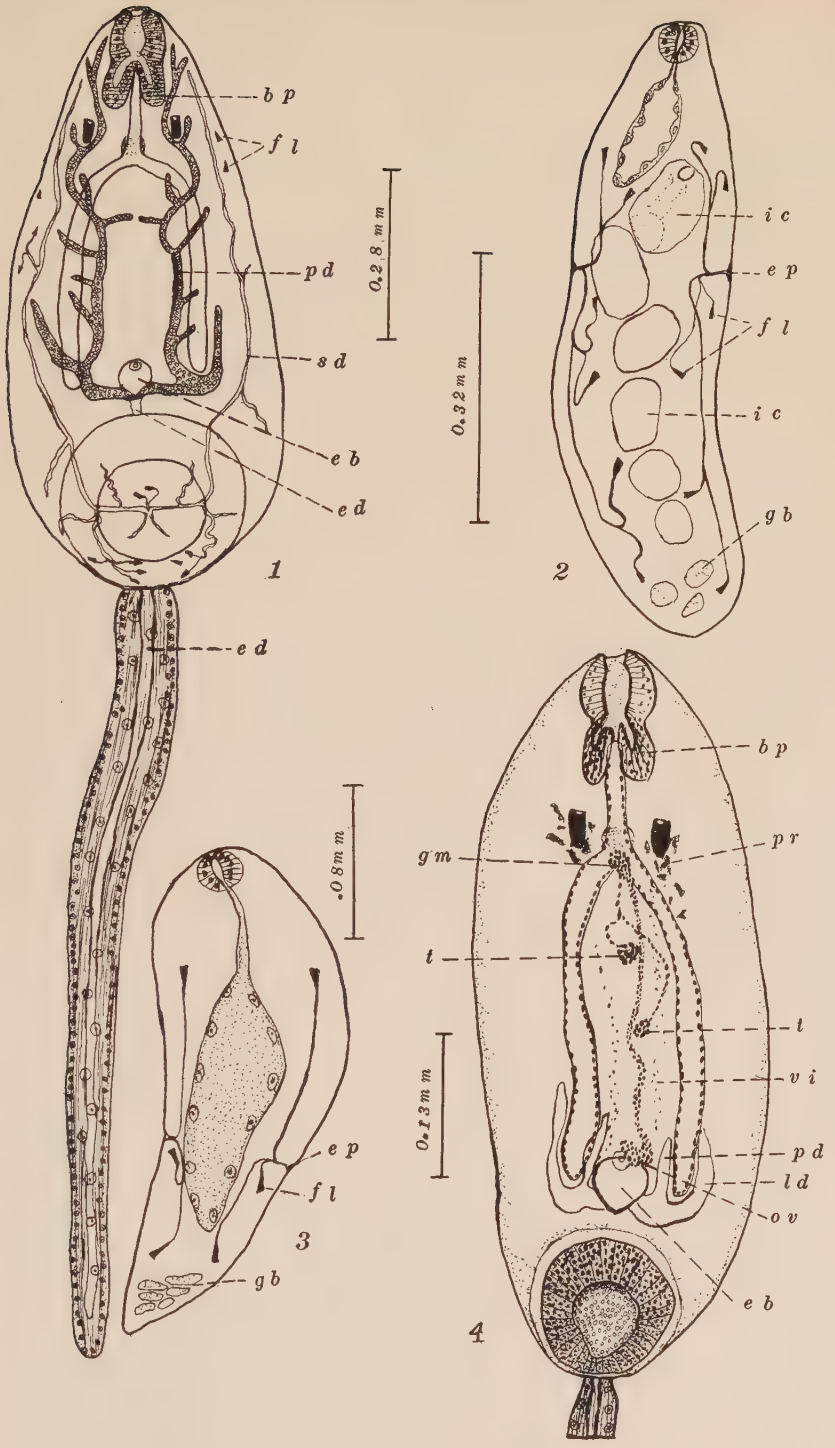

Fig. 1. Cercaria, ventral aspect, showing excretory system. Structure of tail, somewhat contracted, inserted from stained whole mount.

Fig. 2. Mature redia. System of tubules reconstructed from observations on several specimens.

Fig. 3. Immature redia.

Fig. 4. Cercaria, optical section from whole mount seen from ventral aspect.

to merge with the anterior mass of genital cells. A less well-defined column of cells medial and ventral to each intestinal caecum probably represents the anlagen of the vitellaria.

The excretory system (Fig. 1) drains into a large, spherical excretory vesicle situated a short distance in front of the acetabulum. An excretory pore, guarded by a strong sphincter, opens on the dorsal side of the body in front of the vesicle, and connects with it by a narrow tube. At the postero-lateral angles of the vesicle a pair of large main excretory ducts arise, proceed laterally and turn forward to run anteriorly, medial to the caeca. At a point a short distance behind the intestinal bifurcation they turn laterad, pass ventral to the intestine, and extend forward to the level of the oral sucker. Along its course, each main duct has a number of lateral and medial diverticula, hitherto unknown in amphistome cercariae. The system of branches is bilaterally symmetrical and is uniform in all individuals studied. Since it is the same on both sides, only one side will be described. At the blind end of the caecum, a large branch arises and passes forward lateral to the intestine. Between this branch and the place where the main duct crosses the intestine, three smaller diverticula arise and pass laterally for short distances. Another branch comes off just behind the eyespot to pass forward lateral to that organ, and the main duct then divides into two branches lateral to the oral sucker. Two medial diverticula arise from the main excretory duct at about the middle of its course, one passing mediad and almost meeting the one from the opposite side, and the other proceeding for a short distance forward medial to the gut. The main tubes and the diverticula are all filled with refractile excretory concretions of about uniform size. These structures are present from one end of the system to the other and could be seen to move back and forth in the primary tubes and in the branches in response to the body contractions of the cercaria. These concretions were either entirely absent or present only in small numbers in immature cercariae obtained from crushed snails. The tail vesicle joins the excretory bladder dorsally at its anterior border and passes into the tail, where in immature cercariae it forks and opens to the outside near the end of the tail, but neither the forking nor any openings could be found in the tail of fully mature cercariae. Secondary collecting ducts arise from the main excretory duct anteriorly and pass backward to the acetabular region where they undergo divisions which can be traced to a large number of flame cells. Other secondary collecting tubules and numerous flame cells appear in other regions of the body but their connections and extent could not be traced satisfactorily due to their small size and the great amount of pigment and cystogenous material.

The cercariae emerge from the rediae in an immature state and complete their development within the digestive gland of the snail. On emergence they swim about in the side of the dish toward the light for from one to two hours, and then encyst on vegetation or on the glass of the container, usually near the surface of the water. The cysts are dome-shaped hemispheres and have thick resistant walls. The cercariae emerge between 10:00 A. M. and 2:00 P. M. and appear only on bright days. The number escaping from a single snail in any one day was very small. Although no consistent records of daily numbers were kept, occasional counts were made and these indicate an average of between 10 and 15 cercariae per snail per day. Some days there were none and the highest number counted for one day was 26 from one snail. These counts were made by examining the battery jars in which the snails were kept every half hour and removing with a pipette any individuals found. They were easily seen macroscopically and were always on the light side of the dish and usually near the top. None were ever observed to remain longer than a minute at a level deeper than two inches below the surface of the water, and the cysts deposited on the glass were likewise above this level. They encysted on the glass and in the wrinkles of lettuce leaves or other plants at the surface in about equal numbers.

Since 80 per cent of the newts and 86 per cent of the tadpoles in the pond from which the infected snails were taken harbored *Diplodiscus temperatus,* and since the cercaria and life history (Krull and Price, 1932) of that form were not known at that time, it was thought that *C. poconensis* might develop into adults of *Diplodiscus temperatus.* Accordingly, cysts were fed in various ways to tadpoles known to be uninfected. Tadpoles were placed in jars with loose cysts, some of which were seen to be ingested. Others were forcibly fed with a pipette from 10 to 50 encysted metacercariae. In all, 43 *Rana clamitans* tadpoles were thus exposed, but in every case the results were negative. Examination at various intervals as long as three weeks following ingestion of the cysts, showed no worms and revealed the unopened cysts in various parts of the intestine. In some cases, cysts containing dead metacercariae were found entangled in the cords of fecal material from the tadpoles. In view of these attempts, and in view of the report on *Diplodiscus temperatus* by Krull and Price (1932), the present form is not that species and further life history studies should be carried out using some fish or mammal as the next host.

DISCUSSION

C. poconensis is readily distinguished from all other known amphistome cercariae by the branching of the main excretory ducts and the

presence of 12 flame cells in the redia. Further, no other amphistome cercaria has been described which has buccal pouches and develops in a redia without locomotor appendages. Other minor differences also appear which separate it from all known forms. It fits into neither the Diplocotylea nor the Pigmentata groups proposed by Sewell (1922). Members of the Diplocotylea develop in rediae having locomotor appendages and show no cross-anastomoses or branching of the main excretory channels. Rediae of *C. poconensis* have no locomotor processes and the cercaria shows branching of the excretory ducts. The Pigmentata cercariae develop in rediae without "feet," show cross-anastomosis and some branching of the main excretory ducts, but have no buccal pouches. While the redia of *C. poconensis* is also without "feet" and possesses branched excretory ducts two of which almost meet, it has large buccal pouches. With the exception of *Diplodiscus subclavatus,* for which Looss (1892) mentions 4 flame cells in the redia on one side, and *Diplodiscus temperatus* for which Krull and Price (1932) figure a total of 7, all amphistome rediae hitherto described in either group are characterized by the presence of 6 flame cells, while there are 12 in the redia of *C. poconensis.* In view of its similarity to members of the Diplocotylea group in numerous minor features as well as in the possession of buccal pouches, which are of considerable importance in classification since they carry over into the adult form, it seems advisable at this time to assign *C. poconensis* provisionally to the Diplocotylea group. It is probable that the loss or lack of locomotor appendages in a redia, and the development of excretory diverticula in the cercaria are of less importance in classification than the development of a permanent structure like the buccal pouches. The point might be advocated that the redia is a more primitive stage and therefore its structures are more fundamental in classification than those of a later stage. Further, the excretory system is considered one of the most conservative systems in the trematodes and is therefore valuable in establishing natural relationships. However, branching of the primary excretory ducts occurs in some adult amphistomes, as described by Willey (1935) for *Protocladorchis pangasii,* which also possesses buccal pouches. With respect to these two anatomical features, *Protocladorchis pangasii* is similar to *C. poconensis* and this agreement in form tends to support the inclusion of *C. poconensis* in the Diplocotylea group.

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STUDIES ON *DIENTAMOEBA FRAGILIS* (PROTOZOA). I. OBSERVATIONS WITH SPECIAL REFERENCE TO NUCLEAR STRUCTURE

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I. INTRODUCTION

Dientamoeba fragilis, an intestinal amoeba from man, was first described by Jepps and Dobell in 1918 and since then many observers have recorded it from human beings in various parts of the world. In addition, Hegner and Chu (1930) described this species from wild monkeys of the Philippine Islands and Regandanz (1929) has reported its transfer to rats.

Until recently *Dientamoeba fragilis* has been regarded as an exceptionally rare amoeba which degenerated almost at once when removed from the host. Jepps and Dobell, in 1918, referred to eight cases, including one found by Wenyon, and in 1924 Taliaferro and Becker reported that only 33 cases had been recorded in the literature up to that time. However, some recent studies indicate that this amoeba is more prevalent and more viable outside the host than has been supposed. Svensson and Linders (1934) found 38 cases (51.4%) among 74 institutional patients in Sweden when purgative stools were examined and cultures made in addition to repeated examination of normally passed stools. Wenrich, Stabler and Arnett (1935), examining only a single normally-passed stool for each person, recorded an incidence of 4.3% (45 cases) among 1060 American college students, this incidence being slightly higher than that for *Endamoeba histolytica* (4.1%). The stools in which *Dientamoeba* was found were from 4.5 to 12 hours old (average, 7.75) at the time of examination. In August, 1935, a stool which contained many specimens of this species was kept by the writer at room temperature for two days and examined from time to time. After 24 hours considerable numbers of the amoebae remained and after 48 hours there were still a few survivors. No other protozoa were present in the stool, the observations being checked by fixed and stained slides. The ability of this amoeba to live in feces outside the host for as long as 24 to 48 hours may help to explain the fairly high incidences cited above.

The observations here reported are based upon a study of fixed and stained slides from the feces of 70 persons harboring *Dientamoeba.* The purpose of the present communication is to call attention to a new interpretation of the nuclear structure of this species and to note and inter-

pret certain of the variations in morphology. A more comprehensive report on the whole series of cases is reserved for later publication.

The drawings illustrating this paper were made from slides prepared from six stools of one single host. The variations noted are therefore presumably those to be found within a single strain of this amoeba.

These studies have been aided by grants from the Special Research Fund of the University of Pennsylvania.

II. TECHNIQUE

The slides that have been especially studied were all stained with Heidenhain's haematoxylin. Most of the smears were fixed in various modifications of Schaudinn's and Bouin's fluids. Hollande's and Zenker's fixatives and sublimate-acetic and picro-mercuric mixtures were also tried. After most of these fixatives the nuclei of the amoebae stained fairly well in Heidenhain's, except when treated with Schaudinn's without acetic, in which case the nuclei failed to stain. Bouin's fluid was found to be especially good in the modification consisting of 75 parts of saturated aqueous solution of picric acid, 15 parts of formol and 10 parts of glacial acetic acid. When 5% to 20% of glacial acetic was added to Schaudinn's fluid the subsequent nuclear staining was usually satisfactory. The one-half strength Schaudinn's recommended by Wenrich and Geiman (1933), containing 2% to 2½% of acetic also proved useful. While haemalum, Delafield's haematoxylin and Mallory's phosphotungstic acid haematoxylin were tested as stains, none of them gave a nuclear image as sharp as that obtained with Heidenhain's haematoxylin.

III. DIENTAMOEBA AND ITS NUCLEI

In their original description of *Dientamoeba fragilis* Jepps and Dobell (1918) noted particularly: (1) the small size of the organism, with diameters ranging from 3.5 μ to 12 μ; (2) its active motility in the living state with ectoplasm sharply demarcated from the endoplasm; (3) in stained specimens the alveolar appearance of the cytoplasm, with food vacuoles containing various types of bacteria; (4) the fact that about 80% of the organisms had two similar nuclei of unusual constitution; and (5) the absence of cysts.

The nuclei were described as vesicular, the delicate nuclear membrane, with little or no chromatin upon it, enclosing a clear zone surrounding a fairly large central mass or "karyosome" made up of a group of chromatin granules embedded in a weakly staining matrix. In size the nuclei varied from .8 μ to 2.3 μ in diameter and were proportional to the diameter of the body.

Most of those who have published observations on this amoeba have agreed with the original describers in regard to nearly all the features mentioned. Several observers, however, have emphasized deviations from the conditions originally reported, especially with regard to nuclear structure. Thomson and Robertson (1923) observed a small central "karyosome" within the central mass, surrounded by the other chromatin granules. Wenyon (1926) reported a similar observation and suggested a resemblance to the nuclear structure of the members of the genus *Endamoeba*. Reichenow (1929) also suggested that a small central granule constituted the "Binnenkörper" (= endosome = karyosome) and Kudo (1926) referred to the central nuclear mass as the "nucleolus." Craig (1926) emphasized the tendency for the number of granules in the "karyosome" to be commonly four and of equal size, although he mentions frequent variations both in number and size of the granules.

In the present study, not only have the conditions noted by the above authors been verified for the most part, but even greater variations have been found. As a result of the study of a relatively large series of slides the writer has been led to make an interpretation of nuclear structure that differs somewhat from any of those mentioned.

After referring to the "karyosome" made up of chromatin granules imbedded in a matrix, apparently composed of plastin, Jepps and Dobell state: "Frequently one of these chromatin granules appears larger and more deeply stained than the others" (1918, p. 355). The condition described in the sentence quoted appears to the present writer to be *characteristic* and, with variations to be mentioned, has been found in all cases studied where an adequate number of specimens was available. The larger, more deeply staining granule, so definitely indicated by the original describers, is believed to be the true karyosome, or better, endosome. The other granules of the central mass may therefore be regarded as equivalent to the periendosomal granules in other amoebae, as for example, *Iodamoeba*. The eccentric position of the endosome may be explained as due to the tendency for the central mass to assume a spherical form and for the granules to become approximately equally spaced within this spherical matrix.

As suggested above, the granule here interpreted as the endosome is both larger and more deeply staining than the others in the majority of amoeba, be they either large or small, mononucleate or binucleate (Figs. 1, 3, 5, 9, 11, 14, 15, 16, 18, 19, 20, 21, 22, and 24). In some cases it is difficult to identify this body by its size (Figs. 2, 4, 6, 7, 8, 10, 12, 13 and 17). On the other hand, in many of these latter instances the endosome may be recognized by its greater stainability. In some of the amoeba it is set off from the surrounding nuclear mass by a clear area which is probably a shrinkage space (Figs. 4, 21, 22, 24 and 25) and in some it appears to be exceptionally large and prominent (Figs. 21, 22 and 24).

As noted by Craig (1926), very often there appear to be just four granules within the nucleus. As indicated above, three of these stain less intensely than the endosome (Figs. 1, 2, 3, 4, 9, 10, 11, 15, 16, 18 and 19). In contrast to this it is not uncommon to find more than four granules and in such cases the number is often approximately eight (Figs. 6, 7, 8, 14, 20 and 21). Although such differences in number can usually be found on the same slide, some stools seem to be characterized by having many more amoebae of the multigranular type than others.

It is in the multigranular type of nucleus that one occasionally sees a granule in the center of the group, as noted by Martin and Robertson (1923), Wenyon (1926) and Reichenow (1929). With more than four granules, it would not be surprising if one of them would become placed approximately in the middle of the group in harmony with their tendency to maintain equal distances from each other. Also a granule that is above or below the optical section of the nucleus may be misinterpreted as central in position. But the central granules noted by the authors mentioned above are said to be, and are pictured as being, smaller and less intensely stained than the others. The writer has seen these conditions but in many cases the supposed central granule is too ill defined to be more than an indefinite condensation of the matrix material.

Since the number of granules in the central nuclear mass may vary from four to eight or sometimes more, it is reasonable to suppose that the larger number may result from the multiplication of the original four. At times the endosome appears to divide before the other granules (Fig. 5, lower nucleus); at other times to remain undivided while the rest have multiplied (Figs. 21, 22 and 24). Unless the four granules divided simultaneously, one would expect all numbers between four and eight to occur and that is what can actually be observed. Additional divisions of some of the granules would give numbers greater than eight and such numbers are sometimes seen.

There are also indications that eight granules in a nucleus may possibly be the result of the coalescence of two nuclei each having four granules. Figures 11, 12, 13 and 14, all drawn from one slide, represent a possible series of stages in nuclear fusion. The condition in figure 14, however, could have been derived from division of granules as readily as from fusion of nuclei. Such coalescence of nuclei may be one expression of abnormality or beginning degeneration.

The variations in body size for this amoeba are notable. The range of diameters from 3.5 μ to 12 μ as noted by Jepps and Dobell have been confirmed by subsequent observers. Occasionally, however, one sees individuals with diameters in excess of 12 μ. Figure 23, for example, represents an animal from a fixed and stained slide measuring between

Explanation of Figures

Dientamoeba fragilis from slides stained with Heidenhain's haematoxylin. All drawings have been outlined with the aid of a camera lucida at an initial magnification of × 3000. They have been reduced to about × 2000 in printing.

Fig. 1. Small individual with comparatively large nuclei and typical arrangement of four granules in the central mass. One of these granules is a little larger and more intensely stained and is believed to be the endosome (or karyosome).

Fig. 2. Another small individual with slight differences in the arrangement of granules in the nucleus. In one nucleus the endosome is easily distinguishable, in the other it is not.

Fig. 3. Fairly typical individual with compact arrangement of granules. Post-division desmose visible.

Fig. 4. Individual in which the endosomes are sharply set off from the other parts of the central mass by a clear zone.

Fig. 5. Fairly large individual with nuclei of relatively small size. In lower nucleus endosome appears to be in division. Post-division desmose between the nuclei.

Fig. 6. Individual 14 microns in diameter. Each nucleus has about eight granules, including two endosomes.

Fig. 7. Another individual showing multiple granules well separated. Endosomes in each and small granules in center of the groups.

Fig. 8. Individual with about eight granules in each nucleus. Two endosomes in each and small granules in center of the groups.

Fig. 9. Small individual (4½ μ) with two nuclei and a food inclusion. Endosomes distinct.

Fig. 10. Small individual with finely granular cytoplasm, which stains deeply; endosomes distinct. "Pseudocyst"?

Fig. 11. Individual with two nuclei pressed together (possibly by the large central vacuole); a possible first stage in nuclear fusion.

Fig. 12. Apparent fusion of two nuclei indicated.

Fig. 13. Arrangement of granules suggesting result of nuclear fusion.

Fig. 14. Mononucleate individual with eight nuclear granules including two endosomes, possibly resulting from nuclear fusion. (Figs. 11 to 14 drawn from same slide).

Fig. 15. Mononucleate individual with relatively large clear zone in nucleus; endosome distinct.

Fig. 16. Mononucleate individual with small nucleus; membrane more definite; endosome distinct.

Fig. 17. Small individual with relatively large nucleus; more than four granules; endosome not easily distinguishable.

Fig. 18. Mononucleate individual with finely granular cytoplasm; four nuclear granules, well separated; endosome distinct.

Fig. 19. Mononucleate individual with four nuclear granules, large endosome.

Fig. 20. Mononucleate individual with eight nuclear granules including two endosomes close together.

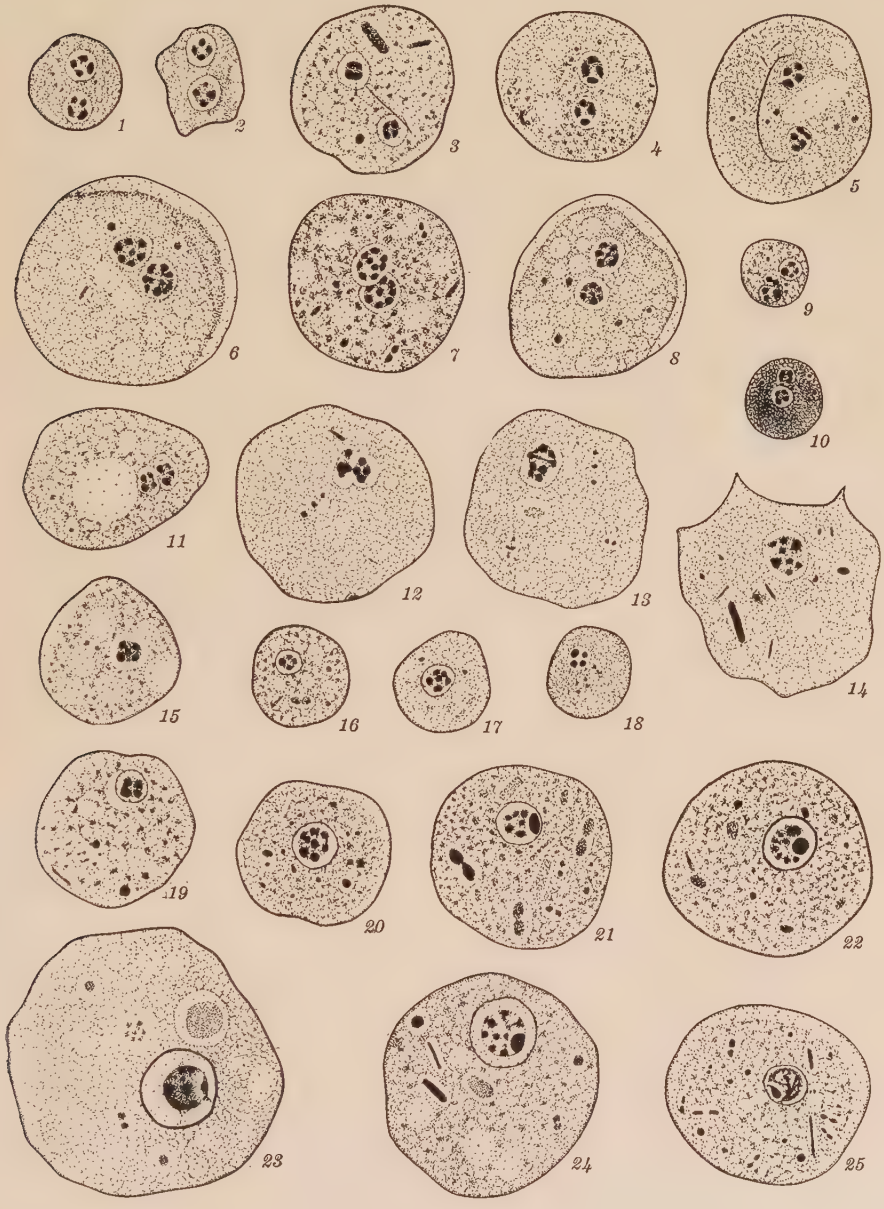
Fig. 21. Large mononucleate individual with more than four nuclear granules and enlarged single endosome set off by a clear zone.

Fig. 22. Large mononucleate individual with granules apparently beginning to fuse. Endosome large and well separated.

Fig. 23. Very large individual (17 + μ) with expanded nucleus; granules fading into matrix; endosome small.

Fig. 24. Large mononucleate individual with nuclear granules tending to lose their identity; endosome large.

Fig. 25. Mononucleate individual with degenerating nucleus; granules are lost in an indefinite network; endosome apparently beginning to dissolve.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17 μ and 18 μ. The majority of these unusually large amoeba are mononucleate although occasionally one finds a binucleate specimen which exceeds 12 μ in diameter, as, for example, the amoeba, 14 μ in diameter, represented by figure 6.

In these larger mononucleate amoebae the nuclei (and sometimes the cytoplasm) appear to be abnormal. The nucleus is usually much enlarged with a more chromatic membrane and the endosome is frequently hypertrophied (Figs. 21, 22 and 24) although it may remain small (Figs. 23 and 25). There are usually more than four granules and they may exhibit various types of degeneration such as fading into the matrix (Fig. 23) or losing their identity in an indefinite network (Fig. 25). In some cases these apparently degenerating nuclei stain faintly and occasionally one finds an individual in which no nuclear structure can be detected. It might be reasonable to suppose that these large mononucleate individuals had lost the capacity for nuclear division while retaining for a time the ability to feed and grow.

Another unusual condition is that found in small and medium-sized individuals, both mononucleate and binucleate, in which the cytoplasm becomes finely and uniformly granular (Figs. 10 and 18). Often these individuals stain more intensely than the more usual type (Fig. 10). Although these peculiar specimens may be degenerate, their nuclei are fairly normal and it is suggested that they might be equivalent to the precystic stages of other intestinal amoebae or even constitute "pseudocysts." No definitive cysts of *Dientamoeba* have been identified in the present study.

IV. SUMMARY

Dientamoeba fragilis is shown to be neither so rare nor so incapable of persistence in feces outside the host as has heretofore been supposed.

The descriptions of this amoeba by Jepps and Dobell and subsequent observers has been confirmed for the most part but a wider range of variation has been described.

The vesicular nucleus contains a central mass ("karyosome" of Jepps and Dobell) which commonly includes from four to eight chromatic granules embedded in a matrix. The more characteristic number of granules seems to be four. One of the granules is usually somewhat larger and more deeply staining than the others and this one is regarded as the true endosome (= karyosome), while the others are thought to constitute the periendosomal granules corresponding to the periendosomal chromatin of other amoebae.

Where there are more than four granules in the central nuclear mass it is suggested that the larger number results from the multiplication of the original four. Evidence is also presented indicating the possibility

that two nuclei, each with four granules, may coalesce to give a nucleus with eight granules.

In some cases one finds larger individuals, commonly mononucleate, with more chromatic nuclear membranes, enlarged endosomes and evidence of degeneration of the nuclear structure. These are thought to exhibit pathological conditions where mononucleate individuals had lost the capacity for nuclear division while retaining for a time the ability to feed and grow.

Attention is called to smaller and medium-sized individuals either mononucleate or binucleate, in which the cytoplasm is finely and uniformly granular instead of alveolar. It is suggested that these may correspond to precystic stages of other intestinal amoebae or perhaps to constitute "pseudocysts." No true cysts were identified.

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AN EXPERIMENTAL STUDY OF INTERNAL AUTOINFECTION WITH *HYMENOLEPIS FRATERNA* IN WHITE MICE[1]

ARNE V. HUNNINEN

There are three possible ways that a definitive host may become infected with the dwarf tapeworm: first, by ingestion of eggs which develop into cysticercoids in the villi and then to adults in the lumen of the intestine (proved by Grassi, 1887); second, by ingestion of intermediate hosts in which the cysticercoids have developed (proved by Bacigalupo, 1928); and third, by the development of worms in the intestine from eggs that have never passed out of the host, *i.e.*, internal autoinfection (not proved).

Chandler (1922) stated that "it seems reasonable to believe that the very heavy human infestations, in which hundreds or thousands of worms may be present, are the result of internal autoinfection." Bacigalupo (1932a) after an extensive survey of the children of Argentina found several cases in which the infestation was very heavy, one having over seven thousand worms. He suggested that the heavy infestations in these cases were due to internal autoinfection. Bacigalupo (1932b) has been unable to infect children directly with the dwarf tapeworm eggs and in 1928 he suggested that in Argentina the usual life cycle is indirect, involving an intermediate host. Therefore, in Argentina, internal autoinfection would introduce a new factor, that is, direct development of the eggs *in situ* after the host has been infected indirectly by means of ingesting intermediate hosts. Here we would have a shifting of the indirect into the direct life cycle. A number of other workers have also suggested that internal autoinfection probably occurs with this parasite but it has never been proved experimentally.

Even if we accept the possibility that the occasional cases in which very heavy infestations are found are due to internal autoinfection, the further question arises whether it is a common or an unusual occurrence and whether it is determined by the physical condition of the host or by individual variation.

Since in other studies (Hunninen, 1935b) on *H. fraterna* in the mouse it has been demonstrated that cysticercoids do not remain for more than six or at most seven days in the intestinal villi after the ingestion of the eggs, it is necessary in order to prove internal autoinfection to demonstrate adults and cysticercoids in the intestine at the same time

[1] From the Department of Helminthology of the School of Hygiene and Public Health of the Johns Hopkins University. The writer is indebted for many helpful suggestions to Dr. W. W. Cort, under whose direction the work was carried out.

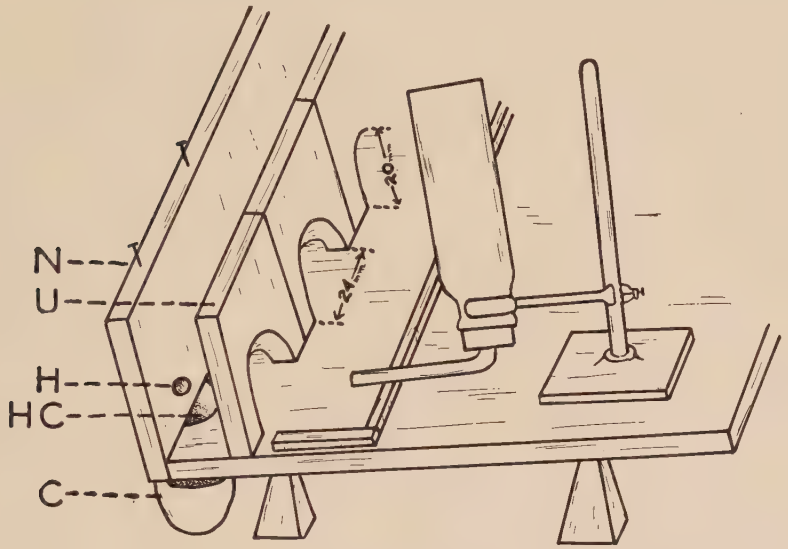

FIG. 1. An apparatus "stocks" devised to prevent infection from outside sources for the purpose of testing internal autoinfection of mice with *H. fraterna*.
H = nail, U = unit of the "stock," H = hole for tail of mouse.
HC = hole for pellets to drop through into the cup (C).

in hosts in which infection from outside sources has been prevented for at least this period of time. In the experiments of this paper a method has been devised to meet these requirements. This was done by placing the mice in "stocks" shown in Figure 1. Before placing a mouse into the "stocks" a piece of adhesive tape was wound around the base of the tail and a string around the tail over the tape. The tail and the string were then pulled through the hole (H) in the backboard and the loose end of the string tied to a nail (N). The unit of the "stock" (U) was then placed over the pelvis of the mouse and with small brads nailed firmly to the baseboard. The dimensions given of the unit of the "stock" are only approximate since it had to be adjusted to the size of the individual mouse. A series of 12 "stocks" were constructed on one baseboard. The attached string, the backboard, and the "stocks held the mice firmly in place under minimum discomfort. The fecal pellets were deposited into a cup (C) through a hole (HC) bored in a convenient place next to the backboard. Food and water were contiually available to all of the mice. It can be seen that the mice in these "stocks" could not possibly become infected from their own feces or from those of the other animals. Upon remaining in the "stocks" for various lengths of time the mice were autopsied, the adults of *H. fraterna* were counted immediately and the examination for cysticercoids was made by a method previously described (Hunninen, 1935a).

In the first experiment six mice were each infected with 3,500 eggs of the mouse strain of *H. fraterna* and 14 days later, that is, just before

they began passing eggs in their feces, all of the animals were placed in the "stocks." Daily examinations of their feces were made. After seven days in the "stocks" the mice were autopsied and examined for adults and cysticercoids. Two of the six mice did not become infected and at autopsy had no adults or cysticercoids. The other four harbored from 5 to 35 adults but no cysticercoids.

The second experiment was carried out with eight mice, all of which received 5,000 eggs and were placed in the "stocks" 14 days after infection. Two of these were autopsied after remaining in the "stocks" for 11 days, two after 19 days, one after 20 days, one after 22 days, and the last two after 37 days. These mice harbored from 1 to 383 adult worms but none of them had cysticercoids.

A third experiment was carried out with six mice which were selected from a naturally infected colony. The length of time they had been infected and the size of their infestations were, therefore, not known. These animals remained in the "stocks" from 11 to 23 days and at autopsy all of them harbored from 2 to 9 adult worms but no cysticercoids.

It is evident, therefore, that internal autoinfection did not occur in the 20 mice in these three experiments. It seems very possible that the normal positions of the adults and cysticercoids in the hosts' intestine are responsible to a great extent for this. In another study (Hunninen, 1935b) it has been shown that the adults are limited to the last 14 cms. of the small intestine in normal mice and that 86 per cent of the cysticercoids are found in the anterior half of the small intestine, the number occurring in the region where the adults are found is very small. If internal autoinfection occurred in mice with normal infestations, the eggs from the worms would have to reach a more anterior location, either by means of reverse peristolsis of the intestine or by the migration of the worms themselves. This, however, appears very improbable in normal mice. Another factor, that is, acquired resistance, appears to play an important rôle in preventing internal autoinfection. It has been shown (Hunninen, 1935) that there is a high degree of acquired resistance produced against a second infection if the mice already harbor worms from a first infection. This resistance is absolute from the fifth to the eighteenth day after the first infection, beyond which it was not tested. Therefore, we can see how difficult it would be to have internal autoinfection in normal mice.

The question arises, then, whether internal autoinfection could occur in mice whose resistance had been lowered. Fortunately the writer encountered four mice suffering severely from diarrhea and intestinal ulcers (probably caused by mouse paratyphoid). These mice were each infected with 3,500 eggs and placed in the "stocks" 14 days later. After

they had remained in the "stocks" for six days one of them died, and since the others were very emaciated and on the verge of death they were also killed. At autopsy it became immediately evident that the worms were abnormally scattered beyond their normal location in the small intestine.[2] The first mouse harbored 100 adults which were spread from the cecum anteriorly for 19 cms. It had one cysticercoid located in the last half of the small intestine. The second mouse harbored 221 adult worms, the most anterior of which was 27 cms. from the cecum, and four cysticercoids all of which were in the last half of the small intestine. The third mouse harbored 104 adults, which were scattered from the cecum anteriorly to 35 cms., but no cysticercoids. The fourth mouse was infested with 75 adults, the anterior of which was found 42 cms. from the cecum, and 193 cysticercoids. In this last mouse the worms were spread practically the entire length of the small intestine. Of the 193 cysticercoids 190 were found in the anterior half of the intestine and 160 in the second fourth. None of the cysticercoids in these three mice were found anterior to the most anterior adult worm. We can see, therefore, that in all of these four sick diarrhetic mice, whose resistance undoubtedly was greatly reduced, the worms were abnormally scattered in the intestine and in three of them there was a clear evidence of internal autoinfection.

From the above experiments the suggestion can be made that internal autoinfection with *H. fraterna* does not occur in normal mice but may occur in mice whose resistance has been decreased by some factor as for example, by bacterial infection. Further, this experimental demonstration of the possibility of the occurrence of internal autoinfection under certain conditions in mice, make it seem very probable that it may occur under similar conditions with *H. nana* in man.

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[2] The average length of the small intestine of a 2½ month-old mouse is 45 cms.

A CHECK-LIST AND HOST-INDEX OF THE GENUS HAEMOPROTEUS

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The frequency with which Haemoproteus parasites are recorded and the widely scattered literature on the subject gives ample justification for a check-list and host-index of the genus. The species are numbered from 1 to 45 and the corresponding number or numbers are placed preceding the author's name in the host-index if the author designated the species found. In the desire for completeness a sincere effort has been made to examine the original literature but in some cases, as indicated, it has been necessary to rely on the statements of other workers. Host records and designated species may have been overlooked and the author would welcome correspondence from investigators who may note omissions or errors in the present work.

Genus Haemoproteus Kruse, 1890

Synonyms: *Laverania* Grassi and Feletti, 1891, *partim*; *Halteridium* Labbé, 1894; *Haemamoeba* Simond, 1901; *Haemocystidium* Castellani and Willey, 1904.

Check-List of Haemoproteus Species

1. *H. agamae*♯ (Wenyon, 1909). In *Agama colonorum,* lizard.
2. *H. alaudae* Celli and San Felice, 1891. In *Alauda arvensis,* skylark.
3. *H. aluci* Celli and San Felice, 1891. In (?) *Syrnium aluco,* owl.
4. *H. beckeri* Roudabush and Coatney, 1935. In *Toxostoma rufum,* brown thrasher.
5. *H. bubonis* Celli and San Felice, 1891. In (?) *Strix flammea,* barn owl.
6. *H. cajali* Pittaluga, 1912. In *Clemmys africana,* turtle.
7. *H. chelidonis* Franchini, 1922. In *Chelidon urbica,* swallow.
8. *H. chelodinae* (Johnston and Cleland, 1909). In *Chelodina longicollis,* turtle.
9. *H. columbae* Kruse, 1890. In *Columbia livia,* pigeon.
10. *H. crumenium* (Hirst, 1905). In *Leptoptilus crumeniferus,* stork.
11. *H. danilewskii* Kruse, 1890. In *Corvus cornix,* grey crow.
12. *H. danilewski* var. *hirundinis* Sergent Ed. and Et., 1905. In *Hirundo* sp., swallow.
13. *H. danilewskyi* var. *tinnunculus* Wasielewski and Wülker, 1918. In *Cerchneis tinnunculus,* falcon.
14. *H. fringillae* Labbé, 1894. In *Fringilla caelebs,* chaffinch.
15. *H. geocichlae* (Cleland and Johnston, 1909). In *Geocichla lunulata,* ground thrush.
16. *H. gonzálezi* (Iturbe, 1921). In *Anolis biporcatus,* lizard.
17. *H. grahami* Shortt, 1922. In *Agama nupta,* lizard.
18. *H. kopki* (Mello, 1916). In *Hemidactylus brookei,* lizard. (a–w).
19. *H. lophortyx* O'Roke, 1929. In *Lophortyx californica,* California partridge.
20. *H. maccallumi* Novy and MacNeal, 1905. In *Zenaidura macroura carolinensis,* mourning dove.
21. *H. mansoni* Sambon, 1908. In *Lagopus scoticus,* red grouse (a–f).

♯ Removed to the genus Plasmodium (See Wenyon, 1926).
(a–f) According to Fantham, 1910.
(a–w) According to Wenyon, 1926.

22. *H. mazzai* Parodi and Niño, 1927. In *Pheucticus aureiventris.*
23. *H. meliornis* (Cleland and Johnston, 1909). In *Meliornis novae-hollandae,* honey-eater.
24. *H. melopeliae* (Laveran and Pettit, 1909). In *Melopelia leucoptera,* dove.
25. *H. mesnili* (Bouet, 1909). In *Sepedon* sp. or *Naja* sp., snake.
26. *H. metchnikovi* (Simond, 1901). In *Trionyx indicus,* turtle.
27. *H. moruony* Mello and Braz de Sa, 1916. In *Copsychus saularis,* magpie robin.
28. *H. najae* (Wenyon, 1909). In *Naja hajae* and *H. nigricollis,* snakes.
29. *H. nettionis* Cleland and Johnston, 1909. In *Nettion castaneum* Eyton, teal.
30. *H. noctuae* Celli and San Felice, 1891. In *Athene noctua,* owl.
31. *H. orizivorae* Anschütz, 1909. In *Orizornis oryzivora,* rice bird.
32. *H. passeris* Kruse, 1890. In *Passer hispaniolenis,* Spanish sparrow.
33. *H. philemon* (Cleland and Johnston, 1909). In *Philemon corniculatus,* honey-sucker.
34. *H. phyllodactyli* Shortt, 1922. In *Phyllodactylus elisae,* lizard.
35. *H. porzanae* Galli-Valerio, 1907. In *Porzana pusilla,* crake.
36. *H. ptilotis* (Cleland and Johnston, 1909). In *Ptilotis chrysops,* honey-sucker.
37. *H. quelae* (Marullaz, 1912). In *Quelea erythrops,* weaver finch.
38. *H. roumei* (Bouet, 1909). In *Cinixys belliana,* turtle.
39. *H. rouxi* Novy and MacNeal, 1905. In *Passer* sp., sparrow.
40. *H. sacharovi* Novy and MacNeal, 1905. In *Zenaidura macroura carolinensis,* mourning dove.
41. *H. scolapaci* Galli-Valerio, 1929. In *Scolopax rusticola,* woodcock.
42. *H. simondi* (Castellani and Willey, 1904). In *Hemidactylus leschenaultii,* gecko.
43. *H. syrnii* (Mayer, 1911). In *Syrnium aluco,* owl.
44. *H. tarentolae* Riding, 1930. In *Tarentola annularis,* gecko.
45. *H. testudinus* (Laveran, 1905). In *Testudo pardalis,* turtle.

Host-Index for the Genus Haemoproteus-Aves

Acanthogenys rufigularis—honey-eater—: Cleland, 1915, 1922.
Accentor collaris Scop.—accentor—: (11) Galli-Valerio, 1902.
Accipiter melanoleucus—hawk—: Hamerton, 1930.
Accipiter minullus—: Adant, 1932.
Accipiter nisus—sparrow hawk—: Ziemann, 1898*; Galli-Valerio, 1919.
Acridotheres tristis—myna—: Maya and David, 1912.
Acryllium vulturium—: Hamerton, 1934.
Actophilus africanus—: Hamerton, 1930.
Aelstrida cinerea—: Leger, A. and M., 1914a; Scott, 1926.
Agelaeus icterocephalus—hang nest—: (11) Plimmer, 1913.
Agelaeus phoeniceus—hang-nest—: Galli-Valerio, 1902.
Agelaius phoeniceus (Linné)—red-winged blackbird—: Opie, 1898.
Aidemosyne cantans—: Scott, 1926.
Aidemosyne malabarica Linné—: Uegaki, 1930a.
Alario alario—finch—: Scott, 1926.
(*Alauda arborea*)—*Lullula arborea* (Linné)—wood lark—: (11) Galli-Valerio, 1902.
Alauda arvensis—skylark—: (2?) Grassi and Feletti, 1890; (2) Celli and San Felice, 1891; Labbé, 1894; Wasielewski, 1896*; Ziemann, 1898*; Galli-Valerio, 1902; (11) Cardamatis, 1909; Doré, 1922; Franchini, 1923.
Alauda cristata (?)—: (11) Sergent, Ed. and Et., 1905.
Alcedo ispida—: (11) Cardamatis, 1909.
Alectoris chukar—: Hamerton, 1929.
Alectoris graeca vera—: Scott, 1928; Hamerton, 1929.
Aluco longimembris—grass owl—: (9) Russell, 1932.

* According to Wenyon, 1926.
** According to Castellani and Chalmers, Manual of Tropical Medicine.

Amadina fasciata—cut-throat finch—: (11) Plimmer, 1912.
Amaurestes fringilloides—: Schwetz, 1931.
Amazona ventralis—parrot—: Hamerton, 1932.
Amblyornis subalaris—bower bird—: (11) Plimmer, 1912.
Ammoperdix griseogularis—: Hamerton, 1931.
Amoperdix heyi—: Scott, 1927.
Amodina fasciata—: Hamerton, 1929.
(*Anas moschata*)—*Cairina moschata*—muscovy musk duck—: Leger, 1918.
Anas sp.—duck—: Schwetz, 1931.
Anastomus lamelligerus—open bill—: Rodhain, *et al.*, 1913.
Anellobia chryoptera—honey-sucker—: (11) Breinl, 1913.
(*Anthus spinoletta japonicus* (Temminck and Schlegel))—*Anthus japonicus*—pipit—: Ogawa, 1911.
Anthus trivialis Linné—tree pipit—: (11) Galli-Valerio, 1902.
Antigone antigone—sarus crane—: (genus ?) Scott, 1926.
Aphelocephala leucopsis—white face—: Cleland and Johnston, 1911.
Ara macao—parrot—: Hamerton, 1930.
Aramides chiricota—: (genus ?) Scott, 1926.
(*Ardea purpurea*)—*Pyrrherodias purpurea*—purple heron—: Rodhain, *et al.* 1913; Franchini, 1924.
Ardeola bacchus—: Mathis and Leger, 1910.
(*Ardeola flavicollis*)—*Depetor flavicollis*—tiger bittern—: Mathis and Leger, 1910.
Ardetta sinensis (Gmelin)—yellow bittern—: Mathis and Leger, 1910; Ogawa, 1911.
Argusianus argus—argus pheasant—: Scott, 1926, 1928; Hamerton, 1929, 1934.
(*Asio accipitrinus*)—*Otus brachyotus*—short-eared owl—: (?) Celli and San Felice, 1891; Ziemann 1898*; Leger, 1918.
Asio flammeus—: Hamerton, 1934.
(*Asio leucotis*)—*Scops leucotis*—: Scott, 1926.
Asio otus—(*Otus vulgaris*—*Strix otus*)—long-eared owl—: Galli-Valerio, 1902; Böing, 1925.
Astur palumbarius—goshawk—: Wasielewski, 1908.
Asturinula monogrammica—hawk—: Rodhain, *et al.*, 1913; (genus ?) Scott, 1927.
Athene noctua—little owl—: Grassi and Feletti, 1890; (30) Celli and San Felice, 1891; Ziemann, 1898*; Schaudinn, 1904*; Sergent, Ed. and Et., 1904; (11) Plimmer, 1913; Wülker, 1919 (?); Franchini, 1924; Scott, 1928.
Balearica ceciliae—crowned crane—: Wenyon, 1909.
Balearica pavonica cecilia—crowned crane—: Scott, 1927; Hamerton, 1934.
Balearica pavonina—crowned crane—: Todd and Wolbach, 1912.
Balearica regulorum—crowned crane—: Rodhain, *et al.*, 1913; Scott, 1926.
Barnardius semitorquata—: (genus ?) Scott, 1927.
Bitis arietans—: Hamerton, 1934.
Botaurus stellaris—bittern—: Böing, 1925.
Brachyspiza strygiceps dabbenei (Hellmayer)—: Mazza, *et al.*, 1928.
Bubo bubo—great eagle owl—: Scott, 1926.
Bubo capensis—owl—: (11) Plimmer, 1912.
Bubo cinerascenus—: Balfour, 1908.
Bubo cinerascens—: Hamerton, 1934.
Bubo lacteus—eagle owl—: (11) Carpano, 1913; Rodhain, *et al.*, 1913; Leger, A. and M., 1914a.
Bubo maculosus—eagle owl—: (11) Plimmer, 1912, (11) 1914.
(*Bubo poensis*)—*Huhua poenis*—owl—: (11) Plimmer, 1912; Scott, 1927.
Bubo virginianus—eagle owl—: Opie, 1898; Galli-Valerio, 1902.
Bubo sp.—eagle owl—: Galli-Valerio, 1902.
Bucco maculatus striatipectus Scl.—: Mazza, *et al.*, 1927.
(*Budytes flavus*)—*Motacilla flava* (Linné)—yellow wagtail—: (11) Galli-Valerio, 1902; (11) Cardamatis, 1909.
Buteo lineatus—hawk—: (11) (?) Novy and MacNeal, 1905a.

(*Buteo vulgaris*)—*Buteo buteo*—common buzzard—: Pfeiffer, 1890; Galli-Valerio, 1902.
(*Buteo vulpinus*)—*Buteo desertorum*—buzzard—: Danilewsky, 1889*.
Bycanistes cristatus—hornbill—: Ross 1911 (?).
Bycanistes sharpei duboisi—hornbill—: Schwetz and Fornara, 1929; Schwetz, 1931.
Caccabis melanocephala—partridge—: (11) Plimmer, 1915.
Caccabis petrosa—Barbary partridge—: (11) Carpano, 1913.
Caereba cyanea—sugar bird—: (11) Plimmer, 1912, (11) 1913.
Calaenas nicobarica—: Scott, 1926.
(*Calliste cyanoptera*)—*Calospiza cyanoptera*—tanager—: (11) Plimmer, 1912; Iturbe and González, 1916.
(*Calliste festiva*)—*Calospiza cyanocephala*—tanager—: (11) Plimmer, 1912.
(*Calliste melanonota*)—*Calospiza melanonota*—tanager—: (11) Plimmer, 1912.
Calliste tricolor Gmelin—tanager—: (11) Plimmer, 1912; Carini and Maciel, 1916.
Caloenas nicobarica—Nicobar pigeon—: Zoological Gardens, London (Wenyon, 1926).
Calopelia puella—Schlegel's dove—: (11) Plimmer, 1912.
Cancroma cochlearia—: Hamerton, 1929.
Caprimulgus europaeus—nightjar—: (11) Cardamatis, 1909; Böing, 1925.
Caprimulgus fossei—nightjar—: Kérandel, 1909.
Caprimulgus rufus Bodd—: Mazza and Fiora, 1932.
Caprimulgus sp.—nightjar—: (11) Sergent, Ed. and Et., 1904, 1905.
Capsiempis flaveola—tyrant bird—: Iturbe and González, 1916.
(*Cardinalis cardinalis*)—*Richmondena cardinalis* (Linné)—cardinal—: Scott, 1927; Hamerton, 1930, 1932.
Cardinalis phoeniceus—finch—: (11) Plimmer, 1917.
Carduelis elegans Steph.—goldfinch—: (11) Galli-Valerio, 1902; (11) Cardamatis, 1909.
Cariama cristata—: Lutz and Meyer, 1908.
Carine noctua—: Galli-Valerio, 1902.
Cassiculus melanicterus—hang-net—: (11) Plimmer, 1912.
Cathartes aura—turkey buzzard—: (11) Darling, 1912.
Cathartes burrovianus—South American turkey vulture—: Scott, 1926.
Catheturus lathami—mound-builder—: Cleland and Johnston, 1911; Cleland, 1915.
Centropus javanicus—Java coul—: Hegner and Chu, 1930.
Centropus monachus—cuckoo—: Leger, A. and M., 1914a.
Centropus rufipennis—cuckoo—: Scott, 1927.
Centropus sinensis—cuckoo—: Mathis and Leger, 1911.
Centropus superciliosus—cuckoo—: Minchin, 1910; Rodhain, *et al.*, 1913.
Cerchneis alopex—kestrel—: Leger, A. and M., 1914a.
Cerchneis sparverius australis (Redgn.)—kestrel—: Mazza, *et al.*, 1928.
Cerchneis tinnunculus—common kestrel—: (13) Wasielewski and Wülker, 1918; Böing, 1925.
Chalcomitra amethystina—black sun bird—: Scott, 1926.
Chamaepelia minuta—grey ground dove—: (genus ?) Scott, 1926.
(*Chanthornus jamaicae*)—*Icterus jamaicai*—hang-nest—: Aragão, 1916.
Chelidon urbica—martin—: (7) Franchini, 1922.
(*Chibia bracteata*)—*Dicruropsis bracteata*—drongo—: (11) Breinl, 1913.
Chlamydodera orientalis—bower bird—: (11) Breinl, 1913.
(*Chloris hortensis*)—*Ligurinus chloris*—greenfinch—: Laveran, 1907*.
Chloris sinica—: Hamerton, 1931.
Chlorophonia pretrei—tanager—: Iturbe and González, 1916.
Chloropis aurifrons—: Scott, 1926; Hamerton, 1932.
(*Chroicocephalus ridibundus*)—*Larus ridibundus*—black-headed gull—: Franchini, 1924.
(*Chrysomitris cucullatus*)—*Spinus cucullatus*—hooded siskin—: (11) Plimmer, 1912.
(*Chrysomitris spinus*)—*Spinus spinus*—siskin—: (11) Plimmer, 1914.

Cichloselys sibiricus—: Hamerton, 1929.
Cicinnurus regius—king bird of paradise—: (11) Plimmer, 1912; Hamerton, 1934.
(*Cinnyris senegalensis*)—*Chalocomitra senegalensis*—sun bird—: Commes, 1918.
Circus aeruginosus—marsh harrier—: Danilewsky, 1889*; Franchini, 1924; Böing, 1925.
Circus macrurus—harrier—: Scott, 1928.
Cittocincla macrura—shama—: (11) Plimmer, 1914, (11) 1917, Scott, 1926.
(*Coccothraustes vulgaris*)—*Coccothraustes coccothraustes*—common hawfinch—: (11) Sergent, Ed. and Et., 1904; Böing, 1925.
(*Coccothraustes vulgaris japonicus* Temminck and Schlegel)—*Coccothraustes japonicus*—Japanese hawfinch—: Ogawa, 1911.
(*Coereba cyanea*)—*Cyanerpes cyaneus*—American creeper—: (11) Plimmer, 1912, (11) 1913.
Colaeus monedula—jackdaw—: Galli-Valerio, 1902.
(*Columba domestica*)—*Columba livia*—pigeon, domestic variety—: (9?) Grassi and Feletti, 1890; Galli-Valerio, 1902; (11) Sergent, Ed. and Et., 1905; (11) Cardamatis, 1909; Leger, 1918.
Columba grisea—: Scott, 1927.
Columba guinea—: Scott, 1926.
Columba jamicae—pigeon—: (9?) Aragão, 1916.
Columba livia—rock pigeon—: (9) Celli and San Felice, 1891; Galli-Valerio, 1902; Sergent, Ed. and Et., 1904; Rodhain, *et al.*, 1913.
Columba palumbus—wood pigeon—: Böing, 1925.
Columba picazuso Tem.—pigeon—: Mazza, *et al.*, 1932.
Columba rufina—pigeon—: Leger, 1918.
Columbina picui—dove—: Mazza and Fiora, 1932.
(*Columbina talpacoti*)—*Chamoepelia talpacoti*—dove—: Iturbe and González, 1916.
(*Columbigallina talpacota*)—*Chamoepella talpacoti*—dove—: (9?) Aragão, 1916.
Conturnix delegorguei—: Rodhain, *et al.*, 1913.
Copsychus saularis—magpie robin—: Mathis and Leger, 1910; (11) Plimmer, 1912, (11) 1916; (27) Mello and Braz de Sa, 1916.
Coracias abyssinicus—roller—: (11) Carpano, 1913; Leger, A. and M., 1914a. Scott, 1926.
Coracias caudatus—roller—: Scott, 1927.
Coracias garrulus—roller—: Danilewsky, 1889*; (11) Cardamatis, 1909; Wülker, 1919.
Coracias indica—Indian roller—: (11) Plimmer, 1912, (11) 1914.
Corvinella corvina—shrike—: Leger, A. and M., 1914a.
(*Corvus americanus*)—*Corvus brachyrhynchus*—crow—: Opie, 1898; Galli-Valerio, 1902.
Corvus corax—(*Corvus corvus*)—raven—: Galli-Valerio, 1902.
Corvus cornix—grey crow—: (11) Kruse, 1890; Galli-Valerio, 1902; Wülker, 1919.
Corvus corone—carrion crow—: Leger, 1913.
(*Corvus frugilegus*)—*Trypanocorax frugilegus*—rook—: Danilewsky, 1898 (?)*; Galli-Valerio, 1902.
Corvus macrorhynchus—crow—: (11) Castellani and Willey, 1904.
(*Corvus macrorhynchus japonensis* Bonaparte)—*Corvus japonensis*—crow—: Ogawa, 1911; Mine, 1914.
Corvus splendens—Indian house crow—: (11) Castellani and Willey, 1904.
Coryllis worcesteri—: Hamerton, 1934.
Coryllis pusillus—: Hamerton, 1930.
Coryphospingue cucullatus—: Mazza, *et al.*, 1927.
Corythaeola cristata—turaco—: Minchin, 1910; (genus ?) Schwetz, 1931.
Coturnix delegorguei—quail—: Rodhain, *et al.*, 1913.
Cracticus destructor—shrike—: (11) Breinl, 1913.
Crateropus bicolor—: Scott, 1927; Hamerton, 1929.
Crateropus striatus—babbler—: (11) Castellani and Willey, 1904.

Crax alector—: Brimont, 1912.
Crax carunculata—: Hamerton, 1929.
(*Crithagra chrysopyga*)—*Serinus icterus*—serin—: (11) Plimmer, 1915.
Croptophaga ani—cuckoo—: Iturbe and González, 1916.
Cryptorhinus afra—Senegal magpie—: (genus ?) Scott, 1926.
Cryptospiza reichenowi ocularis—: Hamerton, 1934.
Crypturus absoletus—: Lutz and Meyer, 1908.
Ctocampsa flaviventris—: Hamerton, 1929.
Cyanerpes cyaneus—: Hamerton, 1933.
Cursorious temmincki—: (genus ?) Scott, 1927.
Cyanocitta cristata—: (11 and 39) Novy and MacNeal, 1905a.
Cyanocorax chrysops (Vieill.)—: Lutz and Meyer, 1908; Mazza and Fiora, 1930.
Cyanops flavifrons—barbet—: (11) Plimmer, 1916.
Cyanoptila bella—: Hamerton, 1931.
Dacilo gigas—"Laughing jackass"—: Welsh and Priestley, 1911.
Dasyptilus pesqueti—: Scott, 1928.
(*Dendrocitta vagabunda*)—*Dendrocitta rufa*—magpie—: (11) Plimmer, 1913.
Dicaeum hirundinaceum—flower pecker—: Cleland, 1915.
Diphyllodes magnifica—: Hamerton, 1932.
Eclectus pectoralis—red-sided parrot—: (11) Plimmer, 1912.
Electus roratus—parrot—: Hamerton, 1929.
Egretta garzetta—: Franchini, 1924.
Elanus caelens—: Hamerton, 1933.
Emberiza cia Linné—bunting—: (11) Galli-Valerio, 1902.
Emberiza citrinella—yellow-hammer—: Wasielewski, 1908.
Emberiza elegans Temminck—bunting—: Ogawa, 1911.
Emberiza melanocephala—bunting—: (11) Cardamatis, 1909.
Emberiza miliaria—bunting—: (11?) Grassi and Feletti, 1890; Galli-Valerio, 1902; (11) Cardamatis, 1909.
(*Emberiza projer*)—*Miliaria miliaria*—common bunting—: Galli-Valerio, 1902.
Emberiza sulphurata—bunting—: Ogawa and Uegaki, 1927.
Entomyza cyanotis—honey-sucker—: Cleland and Johnston, 1911.
(*Eos ricinata*)—*Eos variegata*—lory—: (11) Plimmer, 1915.
Ephippiorhynchus senegalensis—saddle-billed stork—: Wenyon, 1909.
Epops epops upupa—: (11) Cardamatis, 1909.
Eremapteryx leucotis—: Scott, 1928, Hamerton, 1933.
(*Erithacus luscinia*)—*Aedon megarhyncha*—nightingale—: Ziemann, 1898*.
Erithacus rubecula—(*Sylvia rubecula*)—robin—: Wülker, 1919.
(*Erythrocnema unicincta*)—*Parabutes unicinctus*—buzzard—: Iturbe and González, 1916.
Erythropus vespertinus—red-footed falcon—: Danilewsky, 1889*.
Erythrura prasina (Sparrm.)—grass finch—: (11) Plimmer, 1912; Uegaki, 1930a.
Erythrura psittacea—weaver finch—: (11) Plimmer, 1912.
Estrilda cinerea—weaver finch—: See *Aelstrilda cinerea.*
Estrilda coerulescens—: Hamerton, 1931.
Estrilda melpoda—finch—: Hamerton, 1931.
Estrilda troglodytes—: Hamerton, 1934.
Eudynamis cyanocephalus—cuckoo—: (11) Breinl, 1913; Cleland, 1922.
Eulabes intermedia—: Hamerton, 1929, 1930, 1933.
Eulabes javanensis—Malay grackle—: Scott, 1926.
Eulabes religiosa—: Scott, 1928.
Euphonia violacea—tanager—: Iturbe and González, 1916.
(*Euplectes orix*)—*Pyromelana orix*—weaver finch—: (11) Plimmer, 1912.
Euplocamus erythrophthalmus—: Hamerton, 1929.
Eupodotis arabs—Sudan paauw—: Scott, 1926.
Europhona fasciata—: Scott, 1929.
Eurystoma afer—: Hamerton, 1929.
Eurystomus orientalis—broad-billed roller—: Hegner and Chu, 1930.

Euvenura maguari (Gmelin)—stork—: Carini and Maciel, 1916.
Excalfactoria chinensis—Chinese-painted quail—: Scott, 1926.
Excalfactoria lineata—Island painted quail—: (9) Russell, 1932.
Falco aesalon—: (11) Cardamatis, 1909.
(*Falco circactus*)—*Circactus conereus*—serpent eagle—: Rodhain, *et al.*, 1913.
Falco jugger—: Hamerton, 1933.
(*Falco sparverius*)—*Cerchneis sparverius*—American kestrel—: Leger, 1918.
Falco subbuteo—: Hamerton, 1929.
(*Falco tinnunculus*)—*Cerchneis tinnunculus*—kestrel—: Pfeiffer, 1890; Galli-Valerio, 1902; Wasielewski, 1908; (13) Wasielewski and Wülker, 1918.
Falco sp.—falcon—: Wülker, 1919.
Fondia madagascariensis—weaver finch—: Maya and David, 1912; (11) Plimmer, 1913.
Francolinus mulemae—francolin—: Minchin, 1910.
(*Francolinus uluensis schuetti*)—*Francolinus schuetti*—francolin—: Ross, 1911 (?).
Francolinus sp.—francolin—: Rodhain, *et al.*, 1913.
Fringilla caelebs Linné—chaffinch—: (11?) Grassi and Feletti, 1890; (14) Labbé, 1894; Ziemann, 1898*; (11) Galli-Valerio, 1902; Wasielewski, 1908; (11) Cardamatis, 1909; Woodcock, 1909, (14) 1910.
(*Fringilla cannabina*)—*Linaria cannabina*—linnet—: Wasielewski, 1908; (11) Cardamatis, 1909.
(*Fringilla carduelis*)—*Carduelis carduelis*—goldfinch—: (11) Sergent, Ed. and Et., 1904.
(*Fringilla kawahahiba minor* Temminck and Schlegel)—*Ligurinus minor*—Japanese greenfinch—: Ogawa, 1911.
(*Fringilla linota*)—*Linaria cannabina*—linnet—: (11) Sergent, Ed. and Et., 1904.
(*Fringilla petronia*)—*Petronia petronia*—rock sparrow—: (11) Cardamatis, 1909; Leger, 1913.
Fringilla serinus—: (11) Cardamatis, 1909.
(*Fuligula baeri*)—*Aythya baeri*—Baer's duck—: (11) Plimmer, 1912.
Galerita cristata—: (11) Cardamatis, 1909.
(*Gallinago coelestis*)—*Gallinago gallinago*—snipe—: Wülker, 1919 (?).
Gallinago gallinago—(*Gallinago coelestis*)—snipe—: Franchini, 1924.
Gallinago gallinago raddei (Buturlin) (?)—: Uegaki, 1930.
Gallirex chlorochlamys—: Hamerton, 1930.
Gallus gallus—(*Gallus domesticus*)—fowl—: (11) Plimmer, 1913.
Gallus varius—jungle fowl—: (genus ?) Scott, 1926.
Garrulax albigularis—babbler—: (11) Plimmer, 1913.
Garrulus glandarius—common jay—: Danilewsky, 1898* (?); Galli-Valerio, 1902; Coles, 1914; Wülker, 1919; Böing, 1925.
Garrulus japonicus Temminck and Schlegel—jay—: Ogawa, 1911.
(*Garrulax lanceolatus*)—*Laletes lanceolatus*—jay—: (11) Plimmer, 1914.
Garzetta garzetta—little egret—: Franchini, 1924.
(*Geocichla lunulata*)—*Oreocichla lunulata*—thrush—: (15) Cleland and Johnston, 1909.
Geocichla varia (Pallas)—thrush—: Ogawa, 1911.
Geopelia striata—dove—: Maya and David, 1912.
Geranospizias gracilis—hawk—: Iturbe and González, 1916.
Gerygone albigularis—fly-catcher—: Cleland, 1915.
Glareola pratincola—pratincole—: (11) Plimmer, 1913.
Glarus argentatus—: (11) Cardamatis, 1909.
Glaucidium perlatum—owl—: Leger, A. and M., 1914a.
(*Gracula intermedia*)—*Eulabes intermedia*—wattled startling—: (11) Plimmer, 1916.
Grallina picata—magpie lark—: Cleland and Johnston, 1911.
Gryps fulvus—: Scott, 1926.
Grus japonensis—crane—: (11) Plimmer, 1912.
Grus antigone—crane—: Scott, 1928.

Gymnobucco calvus—barbet—: Theiler, 1930.
Gymnorhina leuconota—piping crow—: (11) Plimmer, 1912.
Gymnorhis flavicollis—finch—: (11) Plimmer, 1913.
(*Gypogeranus serpentarius*)—*Serpentarius serpentarius*—secretary bird—: (11) Carpano, 1913.
(*Hadrostomus rufus* (Vieill.))—*Pachyrhamphus rufus*—American chatterer—: Carini and Maciel, 1916.
Halcyon lindsayi—Lindsay's kingfisher—: Hegner and Chu, 1930.
Halcyon senegalensis—kingfisher—: Theiler, 1930.
Haliaetus leucoryphus—Palla's fishing eagle—: Scott, 1926.
Halister girrenera—kite—: (11) Breinl, 1913.
Haliastur indus—: Hamerton, 1931.
(*Hedymeles ludovicianus*)—*Zamelodia ludoviciana*—Rose-breasted grosbeak—: (11) Plimmer, 1912.
Helodromas ochropus—green sandpiper—: Franchini, 1924.
Herodias alba—heron—: Rodhain, *et al.*, 1913.
Herodias egretta—egret—: Hamerton, 1929.
Hirundo aethiopica—: Balfour, 1908.
Hirundo rustica Linné—common swallow—: (11) Galli-Valerio, 1902; Franchini, 1923.
Hirundo sp.—swallow—: (13) Sergent, Ed. and Et., 1905.
Houbara macqueeni—bustard—: (11) Plimmer, 1912.
Hyphantornis cucullatus—weaver finch:—Leger, A. and M., 1914a; Schwetz, 1931.
(*Hyphantornis personata*)—*Sitagra luteola*—slender-billed weaver—: Scott, 1926.
Hyphantornis spilonotus—weaver-finch—: (11) Plimmer, 1912.
(*Hyphantornis taeniopterus*)—*Sitagra intermedius*—weaver bird—: Wenyon, 1909.
(*Hyphantornis textor*)—*Hyphantornis cucullatus*—weaver finch—: (11) Plimmer, 1913.
Hypsipetes amaurotis (Temminck)—bulbul—: Ogawa, 1911.
Ibis aethiopica—ibis—: Minchin, 1910.
Icterus galbula—Baltimore oriole—: (39) Novy and MacNeal, 1905a.
Icterus wagleri—: Hamerton, 1931.
Iole gularis—Philippine bulbul—: Hegner and Chu, 1930.
Ixobrychus sinensis—little yellow bittern—: Hegner and Chu, 1930.
Iynx torquilla—wryneck—: Ogawa, 1911; Wülker, 1919 (?); Franchini, 1924.
Junco hyemalis—slate-colored junco—: Hamerton, 1931.
Ketupa zeylonensis—: Hamerton, 1932.
Lagonosticta senegala (Linné)—weaver-finch—: Commes, 1918.
Lagopus scoticus—grouse—: (21) Sambon, 1908**; Fantham, 1910.
Lamprocolius purpureus—tree starling—: Leger, A. and M., 1914a.
Lamprotreron superba—: Scott, 1927.
(*Lanius bucephalus* Temminck and Schlegel)—*Cephalophoneus bucephalus*—shrike—: Ogawa, 1911.
(*Lanius collurio*)—*Enneoctonus collurio*—red-backed shrike—: Ziemann, 1898*; Wasielewski, 1908; Wüiker, 1919; Böing, 1925.
Lanius excubitor—great grey shrike—: Danilewsky, 1889*; Galli-Valerio, 1902; (11) Sergent, Ed. and Et., 1904 (?); (11) Cardamatis, 1909; Wülker, 1919 (?); Böing, 1925.
Lanius minor—shrike—: Danilewsky, 1889*; Galli-Valerio, 1902; (11) Cardamatis, 1909.
(*Lanius rufus*)—*Phoneus rutilus*—shrike—: Danilewsky, 1889*; Galli-Valerio, 1902.
(*Lanius schach*)—*Cephalophoneus schach*—shrike—: Mathis and Leger, 1910.
Lanius sp.—: (11) Sergent, Ed. and Et., 1905.
Larus cirrhocephalus—gull—: Thiroux, 1911.
Lepidocolaptes angustirostris (Vieill.)—: Mazza, *et al.*, 1927.
Leptoptilus crumeniferus—marabou stork—: (10) Hirst, 1905; Scott, 1926, 1928.
Leptoptilus sp.—stork—: Mathis and Leger, 1910.

Limnocorax niger—: Hamerton, 1929, 1930.
Liothrix luteus—: Hamerton, 1929.
Lissotis melanogaster—(Otis melanogaster)—bustard—: Todd and Wolbach, 1912.
Loriculus galgulus—hanging parrakeet—: (11) Plimmer, 1912, (11) 1913, (11) 1914, (11) 1915.
Lophortyx californicus (Shaw)—California partridge—: (19) O'Roke, 1929.
Loriculus indicus—Ceylon loriquet—: (genus ?) Scott, 1926.
Lorius domicella—lory—: (11) Plimmer, 1912.
Lorius flavopalliatus—lory—: (11) Plimmer, 1917.
Lorius garrulus—lory—: Scott, 1927.
Loxia curvirostra—crossbill—: Wasielewski, 1908; (11) Plimmer, 1913.
(*Luscinia phoenicurus*)—*Phoenicurus phoenicurus*—redstart—: Sjöbring, 1912*.
Luscinia sp.—nightingale—: (11) Sergent, Ed. and Et., 1904.
Lynx torguilla Linné—: Ogawa, 1911.
Lyurus tetrix—blackgame—: Böing, 1925.
Machlolophus xanthogenys—yellow-checked tit—: (11) Plimmer, 1912.
Malimbus nitens—Gray's malimbe—: (genus ?) Scott, 1926.
Machetornes rixosa (Vieill.)—fly-catcher—: Mazza and Fiora, 1932.
Megaloprepia magnifica—: Scott, 1927.
Megalurus gramineus—warbler—: (11) Breinl, 1913.
Melaenornis edoloides—fly-catcher—: Commes, 1918.
Meleagris gallopavo—turkey, domestic—: Macfie, 1916.
Melierax gabar—chanting goshawk—: Leger and Husnot, 1912.
Melierax polyzonus—: Hamerton, 1929.
Meliornis novae-hollandae Lath.—honey-sucker—: (23) Cleland and Johnston, 1909.
Melithreptus atricapillus Lath.—honey-sucker—: Cleland and Johnston, 1910.
Melithreptus brevirostris—honey-sucker—: Cleland, 1922.
Melithreptus validirostris—honey-sucker—: Cleland, 1915.
Melittophgus gularis gularis—bee-eater—: Theiler, 1930; Strong and Shattuck, 1930.
Melopelia leucoptera—dove—: (24) Laveran and Pettit, 1909.
Melophus melanicterus—bunting—: (11) Plimmer, 1913.
Melophus melanocephalus—: Scott, 1927.
(*Melospiza fasciata*)—*Melospiza melodia*—American song sparrow—: Opie, 1898; Galli-Valerio, 1902; (11) Novy and MacNeal, 1905a.
Melospiza georgiana (Lath.)—swamp sparrow—: Opie, 1898; Galli-Valerio, 1902.
Merops albicollis—bee-eater—: Minchin, 1910.
Merops apiaster—European bee-eater—: (11) Sergent, Ed. and Et., 1904; (11) Cardamatis, 1909.
Merops nubicus—bee-eater—: Balfour, 1908.
Merops ornatus—bee-eater—: Cleland and Johnston, 1911.
Merula migratoria—robin—: (?) (11) Novy and MacNeal, 1905a.
(*Merula vulgaris*)—*Merula merula*—blackbird—: Coles, 1914.
Mesia argentauris—babbler—: (11) Plimmer, 1913.
Microeca fascinans—fly-catcher—: Cleland and Johnston, 1911.
Milvago chimachima—carrion hawk—: Iturbe and González, 1916.
(*Milvus migrans*)—*Milvus korschun*—black kite—: Danilewsky, 1889*; Galli-Valerio, 1902; Rodhain, *et al.*, 1913.
Milvus milvus—common kite—: Böing, 1925.
Mimocichla rubripes—: Scott, 1928.
Moineau du Tonkin—: Mathis and Leger, 1910.
Molothrus badius (Vieill.)—cowbird—: Mazza, *et al.*, 1927.
(*Monedula turrium*)—*Colaeus monedula*—jackdaw—: Danilewsky, 1889.*
Munia atricapilla—: (genus ?) Scott, 1927.
Munia cabanisi—Cabanis's weaver—: (9) Russell, 1932.
Munia topela (Swinhoe)—weaver-finch—: Mathis and Leger, 1910; Uegaki, 1930a.
Muscicapa grisola—spotted fly-catcher—: (11) Cardamatis, 1909.

Muscivora tyrannus Linné—tyrant bird—: Carini and Maciel, 1916.
Musophaga rossae—turaco—: Minchin, 1910.
Mycteria americana—: Lutz and Meyer, 1908; Hamerton, 1930.
Myiagra nitida—fly-catcher—: Cleland and Johnston, 1910.
Myioborus brunneiceps (Waf. and DO'rb.)—warbler—: Mazzo and Fiora, 1932.
(*Myiozetetes texensis*)—*Myiobetetes similis*—tyrant bird—: Iturbe and González, 1916.
Myristicivora bicolor—: (genus ?) Scott, 1926.
Myzantha flavigula—: Cleland and Johnston, 1911.
Myzantha garrula—honey-sucker—: Cleland and Johnston, 1911.
Myzomela sanguineolenta—honey-sucker—: Cleland and Johnston, 1911; Cleland, 1915.
Necrosyrtes monachus—hooded vulture—: Scott, 1926; Hamerton, 1934.
(*Nectarina platyura*)—*Hedydipna platyura*—sunbird—: Leger, A. and M., 1914a.
(*Neophron monachus*)—*Necrosyrtes monachus*—hooded vulture—: Balfour, 1908; Todd and Walbach, 1912; Leger, A. and M., 1914a.
Netta rufina—: Hamerton, 1931.
Nettion castaneum Eyton—teal—: (29) Johnston and Cleland, 1909a.
Nettopus coromandelianus—cotton teal—: (11) Plimmer, 1915.
Ninox boobook—owl—: Cleland and Johnston, 1910; (30) Breinl, 1913; Cleland, 1915.
Ninox strenua—owl—: Cleland, 1915.
Nothura maculosa—: Scott, 1927.
Notienas maculosa—: Mazza, *et al.*, 1927.
Notophoyx novae-hollandiae—heron—: (11) Breinl, 1913.
Numenius variegatus—whimbrel—: (9) Russell, 1932.
Numida coronata—guinea-fowl—: Scott, 1928.
Numida meleagris—guinea-fowl—: Kérandel, 1909; Todd and Walbach, 1912.
Numida mitrata—guinea-fowl—: Scott, 1928.
Numida ptilorhyncha—guinea-fowl—: Wenyon, 1909; Minchin, 1910; Rodhain, *et al.*, 1913.
Numida sp.—guinea-fowl—: Ross, 1911 (?); Schwetz, 1931.
Nyctanassa violacea—heron—: (genus ?) Zoological Gardens, London (Wenyon, 1926).
Nycteria americana—wood-ibis—: Scott, 1928.
(*Nycticorax gardenia*)—*Nycticorax nycticorax*—night heron—: Lutz and Meyer, 1908.
Nycticorax nycticorax—(*Nycticorax gardenia*)—night heron—: Franchini, 1924.
Nycticorax nycticorax nycticorax Linné—night heron—: Uegaki, 1930.
Nyctomassa violaceus—: (genus ?) Scott, 1926.
Odontophorus capueira—: Lutz and Meyer, 1908.
Oreocichla lunulata—(*Geocichla lunulata*)—thrush—: Cleland and Johnston, 1909.
Oriolus acrorhynchus—Philippine oriole—: Hegner and Chu, 1930.
Oriolus galbula—golden oriole—: (11) Cardamatis, 1909.
(*Oriolus sagittarius*)—*Mimeta sagittata*—Australian oriole—: Cleland and Johnston, 1911.
Ortalis canicollis (Wagl.)—: Mazza, *et al.*, 1930; Mazza and Fiora, 1932.
(*Orthotomus sutorius*)—*Sutoria sutoria*—tailor bird—: Mathis and Leger, 1910.
Oryzornis oryzivora—: See *Padda oryzivora*.
(*Otis maculipennis*)—*Lissotis maculipennis*—bustard—: Ross, 1911 (?).
(*Otis melanogaster*)—*Lissotis melanogaster*—bustard—: Rodhain, *et al.*, 1913.
Otocorys alpectris—: Hamerton, 1929.
Otogyps calvus—: Scott, 1928.
Otusbakkamoena glablipes—: Ogawa and Uegaki, 1927.
Otus choliba (Vieill.)—owl—: Mazza, *et al.*, 1928.
Otus scops—owl—: Hamerton, 1931.
(*Otus vulgaris*)—*Asio otus*—long-eared owl—: Danilewsky, 1889*.

Pachyrhamphus polychropterus Vieill.—American chatterer—: Carini and Maciel, 1916.
(*Padda oryzivora*)—*Munia orizivora*—weaver bird or Java sparrow—: (31) Anschütz, 1909; Woodcock, 1910 (?); Thiroux, 1911; (11) Plimmer, 1915; Scott, 1926; Uegaki, 1930a.
Paeocephalus fuscicollis—brown-necked parrot—: (11) Plimmer, 1912.
Palaeornis cyanocephala—parrot—: Mathis and Leger, 1910.
Palaeornis fasciata—parrot—: (11) Plimmer, 1913.
Pandion haliaetus—osprey—: Danilewsky, 1889*; Galli-Valerio, 1902.
Paradisea apoda—: Hamerton, 1929.
Paradisea raggiana—bird of paradise—: Scott, 1927.
(*Paradisea rubra*)—*Uranornis rubra*—red bird of paradise—: (11) Plimmer, 1916.
Pardalotus melanocephalus—flower-pecker—: Cleland and Johnston, 1911; Cleland, 1915.
Parotia lawesi—bird of paradise—: (11) Plimmer, 1912.
(*Parus ater*)—*Periparus ater* (Linné)—coal tit—: Galli-Valerio, 1902; (11) Cardamatis, 1909.
(*Parus caeruleus*)—*Cyanistes caeruleus*—blue tit—: Wasielewski, 1908.
Parus major—great tit—: Wasielewski, 1908.
(*Parus palustris*)—*Poecile palustris* (Linné)—marsh tit—: (11) Galli-Valerio, 1902.
(*Passer arcuatus*)—*Passer melanurus*—Cape sparrow—: (11) Plimmer, 1912, (11) 1913.
(*Passer chloris*)—*Ligurinus chloris*—greenfinch—: (11) Sergent, Ed. and Et., 1904.
Passer domesticus—house sparrow—: Opie, 1898; Galli-Valerio, 1902, 1911; Wasielewski, 1908; (11) Cardamatis, 1909; Maya and David, 1912; Petrochi and Zuccarini, 1925.
Passer hispaniolensis—Spanish sparrow—: (32) Kruse, 1890; (32 ?) Grassi and Feletti, 1890; Galli-Valerio, 1902.
Passer italiae—Italian sparrow—: (32) Celli and San Felice, 1891; Ziemann, 1898*; Franchini, 1924.
Passer montanus—tree sparrow—: Grassi and Feletti, 1890; Galli-Valerio, 1902; Mine, 1914; Franchini, 1924.
Passer petronia—sparrow—: (11) Cardamatis, 1909.
Passer sp. (?)—sparrow of Tonkin—: Mathis and Leger, 1910.
Passer sp.—sparrow—: (11) Sergent, Ed. and Et., 1904, (11) 1905; (39) Novy and MacNeal, 1905.
Passerina versicolor (Bonaparte)—varied bunting—: Hamerton, 1929.
Pastor roseus—: Hamerton, 1930.
Pavo muticus—peacock—: Hamerton, 1929.
Penelope obscura—: Lutz and Meyer, 1908.
Penelope pileata—red-breasted guan—: Scott, 1926.
Penelope superciliaris Temminck—guan—: Carini and Maciel, 1916.
(*Penthetria laticauda*)—*Coliostruthus laticauda*—weaver-finch—: Plimmer, 1913.
(*Perdix cinerea*)—*Perdix perdix*—: Laveran and Lucet, 1905; Böing, 1925.
Perdix dactylisonans—: (11) Cardamatis, 1909.
(*Pericrocotus elegans*)—*Pericrocotus speciosus*—minivet—: Mathis and Leger, 1910.
Pernis apivorus—: Galli-Valerio, 1902.
Petroeca phaenicia—fly-catcher—: Cleland and Johnston, 1911.
Petronia petronia—: Hamerton, 1934.
Phalacrocorax africanus—cormorant—: Rodhain, *et al.*, 1913.
Phasianus colchicus—*pheasant*—: Böing, 1925.
Pheucticus aureiventris (DO'rb. and Laf.)—: (22) Parodi and Niño, 1927.
(*Philemon corniculatus*)—*Tropidorhynchus corniculatus*—honey-sucker—: (33) Cleland and Johnston, 1909.
Phylloscopus bonellii Vieill.—warbler—: (11) Galli-Valerio, 1902.

Phylloscopus rufus Bechst.—chiffchaff, warbler—: (11) Galli- Valerio, 1902.
(*Pica caudata*)—*Pica pica*—magpie—: Danilewsky, 1889*; Galli-Valerio, 1902; (11) Cardamatis, 1909.
Pionus menstrus—red-vented parrot—: Scott, 1926.
(*Pishornia scops*)—*Scops scops*—scops owl—: Franchini, 1924.
Piranga azarae—: Mazza and Fiora, 1932.
Piranga flava—: Mazza and Fiora, 1930.
Pitangus sulphuratus—tyrant bird—: (11) Plimmer, 1914.
Pitangus sulphuratus bolivianus (Lafr.)—tyrant bird—: Mazza, *et al.*, 1932.
(*Pitangus sulphuratus maximiliani*)—*Pitangus maximiliani*—tyrant bird—: Iturbe and González, 1916.
(*Planesticus albiventer*)—*Merula albiventer*—thrush—: Iturbe and González, 1916.
Platalea laucorodia—: Hamerton, 1930.
Platycercus adelaidae—parrot—: Cleland and Johnston, 1911.
Plesiositagra cucullata—: Hamerton, 1934.
Plesiositagra nigriceps—: Hamerton, 1933.
Ploceus baya (Blyth.)—: Uegaki, 1930a.
Plotus rufus—darter—: Rodhain, *et al.*, 1913.
Poeocephalus fusicollis—parrot—: (11) Plimmer, 1912, (11) 1916.
Polyborus tharus Mol—carrion hawk—: Carini and Maciel, 1916.
Polyplectrum bicalcaratum—peacock pheasant—: (11) Plimmer, 1914.
Polyplectron germaini—peacock pheasant—: Hamerton, 1930.
Pomatorhinus musicus—babbler—: Ogawa and Uegaki, 1927.
Pomatorhinus superciliosus—scimitar babbler—: Cleland and Johnston, 1910.
(*Porphyrio madagascariensis*)—*Porphyrio porphyrio*—purple gallinule rail—: (11) Plimmer, 1912.
Porzana pusilla—Baillon's crake—: (35) Galli-Valerio, 1907.
Porzana pusilla obscura—rail—: Hamerton, 1929.
Pratincola rubetra Linné—whin-chat—: (11) Galli-Valerio, 1902.
Propasser rhodochrous—finch—: (11) Plimmer, 1917.
Pseudogyps africanus—: Scott, 1928.
Pseudotantalus ibis—wood ibis—: Rodhain, *et al.*, 1913; Leger, A. and M., 1914a.
Psittinus incertus—: Hamerton, 1929, 1930.
Psophia crepitans (Linné)—: Brimont, 1912; Scott, 1927.
Pternistes swainsoni—bare-throated francolin—: (11) Plimmer, 1913.
(*Ptilinopus iozonus*)—*Chlorotreron iozona*—fruit pigeon—: (11) Plimmer, 1915.
Ptilinopus melanocephalus—: Hamerton, 1931.
Ptilogenys cinereus—: Scott, 1928.
Ptilonorhynchus violaceus—satin weaver bird—: Scott, 1926; Hamerton, 1931.
Ptilopachys fuscus—partridge—: Todd and Wolbach, 1912.
Ptilopus coronulatus—lilac-crowned fruit pigeon—: (genus ?) Scott, 1926.
(*Ptilopus superbus*)—*Lamprotreron superba*—fruit pigeon—: (9) Breinl, 1913; Scott, 1927.
Ptilotis auricomis—honey-sucker—: (11) Plimmer, 1915.
Ptilotis chrysops Lath.—honey-sucker—: (36) Cleland and Johnston, 1909; Cleland, 1915.
Ptilotis fusca—honey-sucker—: Cleland and Johnston, 1911; (11) Plimmer, 1912.
Ptilotis leilavalensis—honey-sucker—: Cleland, 1922.
Ptilotis penicillata—honey-sucker—: Cleland, 1915; Hamerton, 1933.
Ptilotis plumula Gould—honey-sucker—: Cleland and Johnston, 1910.
Ptilotis sonora—honey-sucker—: Cleland and Johnston, 1911.
Pycnonotus barbatus—bulbul—: Leger, A. and M., 1914a.
Pycnonotus layardi—: Hamerton, 1932.
Pyromelana flammiceps—weaver-finch—: Leger, A. and M., 1914a.
Pyromelana franciscana—weaver-finch—: Commes, 1918; Scott, 1927; Hamerton, 1930, 1931.
Pyromelana hordacea—: Theiler, 1930.
Pyrrhula griseiventris—finch—: Hamerton, 1929.

Pyrrhula pyrrhula—bullfinch—: Scott, 1927; Hamerton, 1929.
Pyrrhula sp.—bullfinch—: (11) Sergent, Ed. and Et., 1904.
Pyrrhuloxia sinuata—: Scott, 1928; Hamerton, 1933.
Pytelia melba—: Hamerton, 1929.
Quele aerythrops—weaver finch—: (37) Marullaz, 1912.
Rallina eurizonoides—Philippine banded crake—: (9) Russell, 1932.
Rallus aquaticus—water rail—: Franchini, 1924.
Rallus caerulescens—rail—: Hamerton, 1929.
Ramphastos toco—toucan—: Iturbe and González, 1916.
Rupornis leucorrhoa—hawk—: Iturbe and González, 1916.
(*Ruticilla phoenicurus*)—*Phoenicurus phoenicurus* (Linné)—common redstart—: (11) Galli-Valerio, 1902.
Sarcidiornis melanota—wattle duck—: Rodhain, *et al.*, 1913.
Sarcops calvus—gray-backed coleto—: Hegner and Chu, 1930.
Sauroptus chloris—: Hamerton, 1932.
Saxicola oenanthe Linné—wheatear—: (11) Galli-Valerio, 1902; (11) Cardamatis, 1909.
Saxicola stambajina—wheatear—: (11) Cardamatis, 1909.
Scolopax rusticola Linné—woodcock—: Ogawa, 1911; Böing, 1925; Scott, 1926; (41) Galli-Valerio, 1929.
Scops bakkamaena malabaricus—scops owl—: (11) Castellani and Willey, 1904.
Scops brasiliana—scops owl—: Lutz and Meyer, 1908; Leger, 1918.
Scops capensis—scops owl—: Rodhain, *et al.*, 1913.
(*Scops giu*)—*Scops scops*—scops owl—: Sergent, Ed. and Et., 1905.
Scops leucotis—scops owl—: (11) Plimmer, 1912; Scott, 1926, 1927.
Scops semitorques (Temminck and Schlegel)—scops owl—: Ogawa, 1911.
Scotopelia bouvieri—owl—: (11) Plimmer, 1912.
Scotopelia peli—owl—: Rodhain, *et al.*, 1913.
(*Sericulus melinus*)—*Sericulus chrysocephalus*—Regent bird—: Scott, 1926.
Serinus flaviventrus—: Scott, 1928.
Serinus icterus—: Balfour, 1908; Scott, 1928.
Sialia sialis—bluebird, thrush—: Scott, 1926, 1927.
Sitta caesia Wolf—nuthatch—: (11) Galli-Valerio, 1902; (11) Cardamatis, 1909.
Spermestes cucullatus—: Hamerton, 1931.
Spermophilus sp.—: Lutz and Meyer, 1908.
Spizaëtus cirrhatus limnaëtus—: Hamerton, 1931, 1932, 1934.
Spizaëtus coronatus—hawk eagle—: Rodhain, *et al.*, 1913.
Sporaeginthus melpodes—: Scott, 1928.
Spreo superbus—superb spreo—: Zoological Gardens, London (Wenyon, 1926).
Steganurus paradisea verruxi—: Scott, 1928.
Streptopelia semitorquata—(*Turtur semitorquata*)—dove—: Scott, 1926.
Streptopelia semitorquata erythrophrys—dove—: Strong and Shattuck, 1930.
Streptopelia vinacea—dove—: Todd and Wolbach, 1912.
Strix flammea—barn owl—: (5?) Celli and San Felice, 1891; Galli-Valerio, 1902; Sergent, E. and Et., 1907; Balfour, 1908; (11) Plimmer, 1912; Leger, A. and M., 1914a.
Strix flammea trimaculata—barn owl—: Kérandel, 1909.
(*Strix otus*)—*Asio otus*—long-eared owl—: Wasielewski, 1908.
(*Sturnella defilippi*)—*Trupialis defilippi*—hang-nest—: (11) Plimmer, 1913.
Sturnus vulgaris—starling—: Labbé, 1894; Galli-Valerio, 1902; Coles, 1914.
Suthora gularis—: Hamerton, 1931.
Surnia sp.—owl—: (30) Sergent, Ed. and Et., 1905.
Sycalis columbiana—finch—: Iturbe and González, 1916.
Sycalis flaveola—finch—: Iturbe and González, 1916.
Sycobrotus strictifrons—: Hamerton, 1930.
Sylphitreron wallacei—: Hamerton, 1932.
Sylphitreron zonurus—: Hamerton, 1932.

Sylvia atricapilla—blackcap warbler—: (11) Sergent, Ed. and Et., 1904; Wasielewski, 1908.
(*Sylvia cinerea*)—*Sylvia sylvia* (Bechst.)—whitethroat—: (11) Galli-Valerio, 1902; (11) Cardamatis, 1909.
Sylvia hortensis—garden warbler—: Sjöbring, 1892*; Wasielewski, 1908.
(*Sylvia hypolais*)—*Hypolais icterina*—icterine warbler—: Sjöbring, 1892*.
(*Sylvia rubecula*)—*Erithacus rubecula*—robin—: (11) Cardamatis, 1909.
(*Sylvia sibilatrix*)—*Phylloscopus sibilator*—wood warbler—: Sjöbring, 1892*.
(*Sylvia trochila*)—*Phylloscopus trochilus*—willow warbler—: Sjöbring, 1892*.
Sylvia sp.—warbler—: Sergent, Ed. and Et., 1904.
Syrnium aluco—tawny owl—: Danilewsky, 1889*; (3?) Celli and San Felice, 1891; Ziemann, 1898*; Galli-Valerio, 1902; Sergent, Ed. and Et., 1905; (43) Mayer, 1911; (11) Plimmer, 1914.
Syrnium nuchale—owl—: Schwetz, 1931.
(*Tadorna tadornoides*)—*Casarca tadornoides*—sheldrake duck—: (11) Plimmer, 1912.
Taenioptera negeta—tyrant bird—: Carini and Maciel, 1916.
Taeniopygia castanotis—: Hamerton, 1931.
Tanagra cyanoptera—tanager—: Hamerton, 1930.
Tanagra episcopus—tanager—: (11) Plimmer, 1912.
Tanagra flava—tanager—: Hamerton, 1931.
Tanagra palmarum—tanager—: (11) Plimmer, 1912; Iturbe and González, 1916.
Tanagra sayaca Linné—tanager—: Carini and Maciel, 1916.
Tetrao urogallus—capercaillie—: Böing, 1925.
Textor niger—: Scott, 1928.
Trachyphornis arnaudi—: Balfour, 1908.
Tharrhaleus jerdoni—Jerdon's accentor—: (11) Plimmer, 1913.
Thraupis bonariensis (Gmelin)—tanager—: Mazza *et al.*, 1927.
(*Thraupis cana*)—*Tanagra cana*—tanager—: Iturbe and González, 1916.
Thraupis olivicyanea—: Hamerton, 1931.
(*Tinnunculus alaudarius*)—*Cerchneis tinnunculus*—kestrel—: Danilewsky, 1889*; (11) Plimmer, 1912; (11) 1914.
(*Tinnunculus cenchris*)—*Cerchneis naumanni*—lesser kestrel—: (11) Plimmer, 1914.
Totanus ocropus—*sandpiper*—: Franchini, 1924.
Totanus eurhinus—Asiatic redshank—: (9) Russell, 1932.
Toxostoma rufum (Linné)—brown thrasher—: (4) Roudabush and Coatney, 1935.
Trichoglossus forsteni—lory—: Hamerton, 1931.
Trichoglossus nigragularis—lory—: (11) Plimmer, 1912.
Trichoglossus nigragenis—lory—: (11) Plimmer, 1916.
Trichoglossus ornatus—lory—: Hamerton, 1931.
(*Tringa atricapilla*)—*Rostratula semicollaris*—painted snipe—: Leger, 1918.
(*Troglodytes parvulus*)—*Anorthura troglodytes* (Koch)—wren—: (11) Galli-Valerio, 1902; (11) Cardamatis, 1909.
Trogon atricollis—trogon—: Iturbe and González, 1916.
Tropidorhynchus corniculatus—(*Philemon corniculatus*)—honey-sucker—: Cleland and Johnston, 1909, 1911; (11) Breinl, 1913; Cleland, 1922.
Turacoena menadensis—: Hamerton, 1933.
Turacus corythraix—white-crested touracou—: (11) Plimmer, 1912.
Turacus donaldsoni—touracou—: Hamerton, 1930.
Turacus erythrolophus—: Hamerton, 1934.
Turacus macrorhynchus—touracou—: (11) Plimmer, 1912.
Turacus macrorhynchus verreauxi—: Hamerton, 1932.
Turacus persa—touracou—: (11) Plimmer, 1912.
(*Turdus fumigatus*)—*Merula fumigata*—thrush—: Iturbe and González, 1916.
(*Turdus fuscatus* Pallas)—*Turdus dubius*—thrush—: Ogawa, 1912.
Turdus merula—thrush—: (11) Cardamatis, 1909.

(*Turdus musicus*) — *Hylocichla musica* — song-thrush — : Danilewsky, 1889*; Wasielewski, 1908; Coles, 1914; Doré, 1921.
Turdus obscurus Gmelin—thrush— : Ogawa, 1911.
Turdus pallidus Gmelin—thrush— : Ogawa, 1911.
Turnix fasciata—Philippine button quail— : (9) Russell, 1932.
(*Turtur auritus*)— *Turtur turtur*—dove— : Sergent, Ed. and Et., 1904; Balfour, 1908.
Turtur chinensis—dove—Ogawa and Uegaki, 1927.
(*Turtur humilis*)—*Onopopelia humilis*—dove— : Mathis and Leger, 1910.
(*Turtur risorius*)—*Streptopelia risoria*—dove— : (11) Cardamatis, 1909.
Tyranus melancholicus (Vieill.)—Kingbird— : Mazza, *et al.*, 1927.
(*Turtur semitorquatus*)—*Streptopelia semitorquatus*—dove— : Rodhain, *et al.*, 1913.
Turtur senegalensis—dove— : Todd and Wolbach, 1912.
Turtur turtur—(*Turtur auritus*)—dove—Franchini, 1924; Böing, 1925.
Tympanistria tympanistria— : Scott, 1927.
Tyto arfaki—owl— : Scott, 1927.
(*Tyto perlata*)—*Strix perlata*—owl— : Iturbe and González, 1916.
Upupa epops—hoopoe— : Danilewsky, 1889*.
Uraeginthus bengalus— : Hamerton, 1932.
Uraeginthus phenicotis— : Scott, 1928.
Urocissa melanocephala occipitalis— : Hamerton, 1934.
(*Urubitinga albicollis* (Lath.)—*Leucopternis albicollis*—hawk— : Brimont, 1909, 1912.
Vanellus vanellus—lapwing— : Böing, 1925.
Vespertilio murinus— : (11) Cardamatis, 1909.
(*Vidua paradisea*)—*Steganura paradisea*—whyda bird— : (11) Plimmer, 1912, (11) 1917.
Vinago delalandii— : Hamerton, 1934.
Vinago nudirostris—fruit pigeon— : Todd and Wolbach, 1912.
Vireo chivi (Vieill.)—greenlet— : Carini and Maciel, 1916.
Xantholaema haematocephala— : Scott, 1927.
Xanthoura luxuosa— : Hamerton, 1931.
(*Xiphocolaptes procerus*) — *Xiphocolaptes promeropirhynchus* — wood-hewer — : Iturbe and González, 1916.
Xyphocolaptes major (Vieill.)— : Mazza and Fiora, 1930.
Yunx torquilla—woodpecker (?)— : Franchini, 1924.
Zenaidura auriculata (Des Murs)— : Mazza, *et al.*, 1927, 1930.
Zenaidura macroura carolinensis—(*Zenaidura macroura*)—mourning dove— : (20 and 40) Novy and MacNeal, 1905, (40) 1905a; (20 and 40) Huff, 1932.
(*Zonotrichia pileata*)—*Brachyspiza pileata*—finch— : Lutz and Meyer, 1908.
Zonotrichia strigiceps dabbenei (Hellmayer)— : Mazza and Franke, 1928.
(*Zosterops caerulescens*)—*Zosterops lateralis*—white-eye— : Cleland and Johnston, 1910, 1911.
Zosterops japonica Temminck and Schlegel—white-eye— : Ogawa, 1911.
Zosterops palpebrosa peguensis—white-eye— : Ogawa and Uegaki, 1927; (genus ?) Uegaki, 1928.
Zosterops simplex—white-eye— : Mathis and Leger, 1910.

Reptilia

Agama colonorum— : (1♯) Wenyon, 1909; Strong and Shattuck, 1930.
Agama nupta— : (17) Shortt, 1922.
Anolis biporcatus— : (16) Iturbe, 1921.
Chelidina longicollis— : (8) Johnston and Cleland, 1909, (8) 1912.
Chelodina oblonga— : Johnston and Cleland, (8) 1912.
Chrysemys picta—painted terrapin— : Plimmer, 1912.
Cinixys belliana—Bell's cinixys— : (38) Bouet, 1909.
Cinixys erosa—two-eroded cinixys— : Plimmer, 1912.

Cinixys homeana—Home's cinixys—: Plimmer, 1912.
Clemmys africana—: (6) Pittaluga, 1912.
Emydura krefftii Gray—: (8) Johnston and Cleland, 1910, (8) 1912.
Hemidactylus brookei—: (18) Mello, 1916.
Hemidactylus leschenaultii—: (42) Castellani and Willey, 1904; (42) Robertson, 1908; Dobell, 1910.
Naja nigricollis—spitting cobra—: (28) Wenyon, 1908; (28) Macfie, 1919; Scott, 1928.
Naja sp.—: (25) Bouet, 1909.
Phyllodactylus elisae—lizard—(34) Shortt, 1922.
Sepedon haemachates—: Leger, A. and M., 1914.
Sepedon sp.—: (25) Bouet, 1909.
Staurotypus triporcatus—three-tailed terrapin—: Plimmer, 1912.
Tarentola annularis—gecko—: (44) Riding, 1930.
Testudo paradalis—: (45) Laveran, 1905.
(*Trionyx indicus*)—*chitra indica*—: (26) Simond, 1901.

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RESEARCH NOTES

A NOTE ON AN UNDETERMINED PARASITE OF THE ALBINO RAT

A histological study of the digestive tract of rats which had been infected with the coccidium *Eimeria miyairii* revealed the presence of another intra-cellular parasite. Any possibility of its being a stage in the life-cycle of the coccidium was excluded by its discovery in animals which were definitely known never to have experienced a coccidial infection. Examination of 20 rats from the same colony showed the incidence of infection to be 100 per cent. The writers have observed these parasites in rats from three other colonies on the campus at Iowa State College.

The spherical, ovoid or sometimes irregular bodies of this organism have been found in the stomach, the small intestine, the caecum and the large intestine. The parasites are to be found in greatest abundance within the columnar epithelial cells near the base of the intestinal glands. They undergo a period of growth which often causes the nucleus of the host cell to become indented on one or both sides until it appears reniform, (Fig. 1, D—an *in situ* view of the parasite) or club shaped

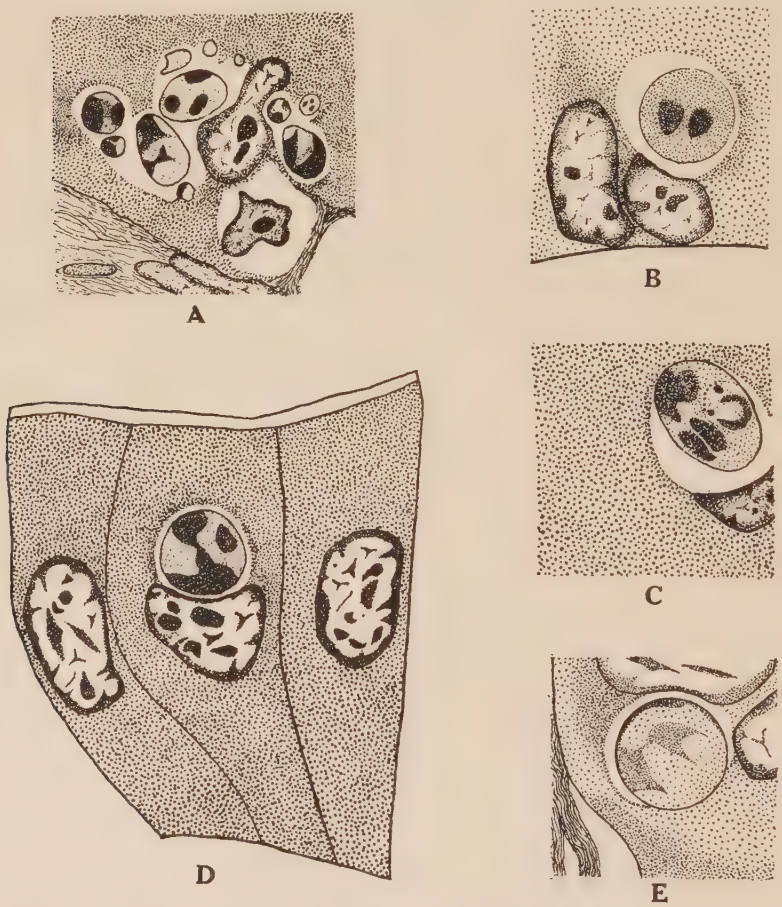

FIG. 1. Parasites in the epithelial cells of the small intestine (A, B, D, E) and the large intestine (C). ×1850.

(Fig. 1, A—from small intestine). In some cases the entire nucleus is displaced to one side of the parasitized cell (Fig. 1, B—from small intestine). By no means, however, are these organisms confined exclusively to the epithelial cells of the intestinal glands, for they are sometimes seen half way out toward the tips of the villi, in the tunica propria, in the muscularis mucosae, and free in the lumen of the intestine. This description of their location in the small intestine does not apply to the rest of the digestive tract, since they are found, in the stomach, to be situated much nearer the lumen (approximately one-fourth the distance from the lumen to the muscularis), while in the caecum and large intestine they seldom penetrate deep into the glands but they are commonly found in the epithelial cells nearest the lumen. They are also found in the lymph glands of the caecum.

All observations of this parasite were made from tissues fixed in Zenker's fixing fluid, stained with methylene blue and counter-stained with eosin. Immediately surrounding the organism is a clear area which, in life, was probably filled with fluid (Fig. 1, C—from large intestine). The smallest form that could be found measured 0.63 μ and stained a uniform pink, while others measured up to 7.87 μ. Organisms of approximately 1.5 μ and over were found to take up, in addition to the eosin, a varied amount of methylene blue. In the drawings, the heavier stippling indicates the methylene blue while the lighter stippling indicates the eosin. The darker areas suggest, by their staining reaction, a nuclear character. There is, however, a noticeable lack of uniformity in shape and size of these structures (Fig. 1, A). Reproduction in this organism is not yet understood, although it seems to include both binary and multiple fission. (Fig. 1, E—from small intestine).

So far as the authors are able to determine, this is a hither-to undescribed parasite. Due to the incompleteness of our knowledge of this organism, its true systematic position is problematical. It seems doubtful that it is a yeast or a mold, since neither reproduction by budding nor mycelia were found. Chatton and Blanc (1917) published figures of schizonts from *Toxoplasma gondii* which have a very close resemblance to certain stages of the parasite found in the rat. No distinct merozoite formation, however, has been observed. Furthermore, in *Toxoplasma* infections, the organisms are reported to occur in abundance in the liver and spleen. Examination of these organs in rats has revealed none of the parasites. As is sugguested above, the final placing of this organism in its correct systematic position depends upon a more complete knowledge of its method of transmission, its method of reproduction, and other phases of its life cycle. Since the taxonomic affinities of this microorganism are so obscure, we are not proposing a name for it at the present time. It is indeed possible that the forms are not organisms at all, but only degenerate stages of lymphocytes like those seen by Mallory (1898, Jour. Exp. Med., 3: 611–638) in endothelial cells in typhoid fever in man. This, however, does not seem very probable.—N. F. Morehouse and E. R. Becker, *Iowa State College.*

THE PROBABLE METHOD OF INFECTION OF THE FOUR-TOED SALAMANDER WITH THE PROTOZOON, *HAPTOPHRYA MICHIGANENSIS*

In an attempt to determine the method of infection of the four-toed salamander *Hemidactylium scutatum* (Schlegel) with the astomatous ciliate of its intestinal tract, *Haptophrya michiganensis* Woodhead, a group of salamanders raised in the laboratory was used. The salamanders were maintained together until removal for actual experimental purposes in order that their environmental conditions would be identical. Finally one was chosen at random and after metamorphosis examined as a control. It was found negative for *Haptophrya.*

Specimens of *Haptophrya* were placed in a small dish containing one larval salamander. The salamanders were attracted by the motions of the protozoa and readily swallowed them. No other type of entrance into the salamander was observed. From the structure of the parasite described by Woodhead (Jour. Parasit., 14: 177–182, 1928) and Bush (Univ. of Cal. Pub. in Zool., 39(12): 251–276) only passive entrance could be expected, although in several cases it was noticed that the ciliates attached themselves to some external part of the salamander.

With but one exception, examinations were made of serial sections of the entire salamander stained in iron haematoxylin. Specimens of *Haptophrya* were found in the intestine, attached by the anterior sucker to the epithelial wall in the usual manner.

Nine salamanders were used, one of the number being kept as a control. Seven days after exposure, two salamanders were killed and found to be, one negative, one positive. Two others were killed nineteen days after exposure. Five days and again three days before killing they were given more of the protozoans. Again one was negative and the other positive. One salamander killed after forty-one days was also negative. A salamander killed after forty-five days, with a second exposure fifteen days before killing proved positive. This individual had metamorphosed as had also the control specimen.

The breeding habits of the adult female salamanders would promote this type of infection. They remain on their nests until after the eggs are hatched and have been observed on empty nests (Blanchard, Copeia, 3: 137–138, 1934). The ciliates are shed in the fecal matter in a non-cystic normal condition and move about in the water. The young can eat these and be infected by them. The infection thus secured is carried through metamorphosis into the adult.—A. E. Woodhead and F. Kruidenier, *Zoology Department, University of Michigan.*

EXPERIMENTAL *TRICHOMONAS FOETUS* INFECTION IN SHEEP

Five non-pregnant ewes injected intravaginally with vaginal washings from a heifer infected with *Trichomonas foetus* (Riedmuller) all became infected within five days after the inoculation. They have remained irregularly positive for over five months to date. During the first month, they had a slight diffuse vaginitis and an abnormally abundant discharge which was generally clear mucus but on occasion was purulent. These symptoms abated within six weeks. One sheep was killed a month after inoculation. No gross pathology of the genitalia was obvious but organisms were found in abundance in the vagina, cervix, and both horns of the uterus. A ram kept in the same stall served the sheep frequently as evidenced by the occurrence of spermatozoa in the vaginal contents of the sheep, but in spite of careful search, no organisms were found in prepucial flushings for two months. The ram was injected in the prepucial space with a culture of bacteria free *T. foetus,* but to date no organisms have been recovered from the host animal. These observations add one more experimentally infectible host for *T. foetus.* The flagellate infection of sheep clinically resembles the low grade temporary infection of heifers. While it is not known that this infection might be venereally transmitted to sheep in nature, these findings suggest that a search be made for this organism particularly in instances of breeding difficulties.—Justin Andrews, *Johns Hopkins School of Hygiene and Public Health* and Charles W. Rees, *U. S. Bureau of Animal Industry.*

REPORT ON THE PREPARATION OF AN HISTOLYTIC FERMENT PRESENT IN THE BODIES OF CERCARIAE

It has long been postulated that certain cercariae secrete from glands in their bodies an histolytic substance which aids in their penetration of the integument of hosts. The writer had access to large numbers of the cercariae of *Diplostomum flexicaudum* last summer at the University of Michigan Biological Station at Douglas Lake and undertook to extract a substance from their bodies which would have a digestive effect on host tissue. This cerceria actively penetrates the integument of the common sucker and several abnormal hosts including frog and toad tadpoles. The life cycle of this form was worked out by Van Haitsma (Mich. Acad. Sc., pp. 483–516, 1930).

An extract of these cercariae was prepared in the following manner. Snails, *Stagnicola emarginata angulata* (Sowerby) known to be infected with *D. flexicau-*

dum were kept in individual half-pint milk bottles. Since the cercariae emerged in greatest numbers in the morning, the water containing a heavy concentration of larvae was poured off from about a dozen snails in the early afternoon, care being taken to keep it free of debris. This was centrifuged to concentrate the cercariae, which were then washed with distilled water by adding it to the tube after most of the water had been poured off and centrifuging again. This process was repeated three times and a fair-sized bolus of cercariae remained in the bottom of the tube when the final supernatant wash water was poured off. The cercariae were then poured into a mortar and ground thoroughly with a pestle until they were completely macerated. The resulting material was kept overnight in a refrigerator and filtered the next morning with filter paper to remove the largest particles. The filtrate was whitish, slightly viscous, and homogeneous, and its amount depended on the degree of concentration before maceration. Usually about four or five cubic centimeters were easily obtainable. A saline extract can be made by washing with normal saline instead of distilled water.

Small pieces of tadpole tisue, two to three millimeters in size, from the muscle, skin, and tip of the tail were placed in a one-inch stender dish containing enough extract to cover them, and similar pieces of tissue were also placed in a dish containing distilled water or saline as controls. The dishes were kept in an ice-box and examined three hours later with a low power dissecting microscope. Differences between the tissues were at once apparent. The tissues which had been in the cercarial extract appeared more transparent and slightly swollen, and the margins were more frayed and loosened than the controls. About the skin there was a gelatinous filmy layer, and the melanophores stood out sharply against the loosened tissue. When the dish was slightly agitated shreds and particles came away and settled to the bottom. The muscle showed fibers distinctly thinner and separated from each other. These changes were not present in the controls. Sections for microscopic study were made and showed equally striking changes (*cf.* Figs. 1 and 2). In the tissues acted on by the cercarial extract the nuclei were barely discern-

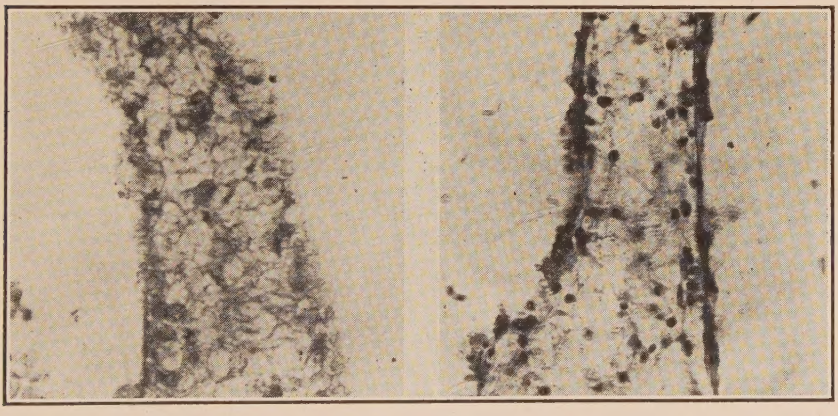

Fig. 1 (left). Photomicrograph magnified about 400 times, of fin of frog tadpole tail which was in aqueous extract of cercariae of *D. flexicaudum* for three hours, showing karyolysis and complete disintegration of epithelium.

Fig. 2 (right). Photomicrograph, magnified about 400 times, of fin of frog tadpole tail which was in distilled water for three hours as control showing intact nuclei and only moderate disintegration of epithelium.

able. They were faded and enlarged and showed marked karyolysis and in certain areas karyorrhexis. This change was more marked on the margins of the pieces of tissue. The stroma which is prominent in the fin of the tail of the tadpole was quite intact and normal.

The extract thus prepared was basic to litmus paper and neutral red dye. Bacterial action was eliminated from this digestive process by refrigeration of the material before filtering and during the histolysis. Heating the extract for half an hour between sixty and eighty degrees Centigrade did not impair its ability to cause lysis of the tadpole tissue.—Dorland J. Davis, *University of Michigan Biological Station and Department of Helminthology, Johns Hopkins School of Hygiene and Public Health.*

AN ABNORMAL *CYSTICERCUS CELLULOSAE* FROM A HUMAN BRAIN

From the complete necropsy of a woman, aged 29 years, eight cysticerci were recovered. All were found in the brain. Four of these were studied. One was found to be a normal *Cysticercus cellulosae* with 4 suckers and with 24 hooks on the rostellum. Two of them were without scolex. The other had a scolex with 4

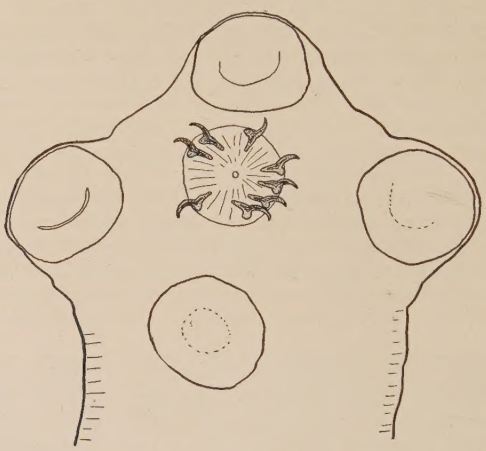

Fig. 1. Scolex of *Cysticercus cellulosae* with only 8 hooks.

suckers but with only 8 hooks on the rostellum. Although malformations of the cysticerci of this species are commonly known to include the absence of rostellum and hooks, and double formation with 6 suckers, we have found no record of a *Cysticercus cellulosae* with only eight hooks.—Clay G. Huff and Paul C. Bucy, *University of Chicago.*